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# Gènes immunitaires du CMH chez un oiseau monogame : fitness et stratégies de reproduction

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*À Papy*

*Un seul être vous manque et tout est dépeuplé*

Alphonse de Lamartine, **L'isolement**, 1820

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# INTRODUCTION

## Sexual selection and genetic quality

"It is certain that the females occasionally exhibit, from unknown causes, the strongest antipathies and preferences for particular males" (Darwin, 1874). When it comes to reproduction, explaining on what criteria individuals base their decisions[1] (e.g., mate choice, investment in offspring) has received considerable attention since decades (Hamilton & Zuk, 1982; Moller & Jennions, 2001; Trivers & Willard, 1973). Such reproductive decisions may depend on the gain of direct, material benefits that are important for successful breeding. For instance, mate preference has been shown to depend on food provisioning or protection against predators (Hoelzer, 1989; Moller & Jennions, 2001). However, absence of direct benefits in many species in which individuals still express a preference has led biologists to propose that reproductive decisions may also depend on the acquisition of indirect, genetic benefits that confer fitness advantages to offspring (Trivers, 1972). Several mechanisms have been proposed for the acquisition of such genetic benefits.

*Genetic benefits and where to find them*

The genetic quality of mates can be absolute, such that the preferred ones would be those carrying "good genes", i.e. good alleles affecting offspring fitness independently of the chooser's genotype (Kokko, Brooks, Jennions, & Morley, 2003; Neff & Pitcher, 2005). Preference for mates carrying good alleles would increase offspring fitness by conferring them reproductive and/or survivorship advantages through the transfer of these good alleles.

[1] Terms such as « decision » or « choice » do not necessarily imply sophisticated cognitive mechanisms and refer here, and throughout the thesis, to changes in traits (behavioral, physiological or other) when animals face several alternatives (environments, mates) that differ in their consequences on fitness (Danchin et al., 2008).

Evidence of mate preference for “good genes” has been shown in numerous species (Cox & Calsbeek, 2010; Eizaguirre, Yeates, Lenz, Kalbe, & Milinski, 2009; Ekblom et al., 2004; Fisher, Double, Blomberg, Jennions, & Cockburn, 2006; Hasselquist, Bensch, & vonSchantz, 1996). The genetic quality of mates can also be relative, such that offspring quality does not result from additive genetic quality of its parents but from the combination of the father’s and the mother’s genotypes (i.e. "compatible genes"; Trivers, 1972; Zeh & Zeh, 1996, 1997). For instance, a preference for genetically dissimilar mates over the whole genome or at specific loci would increase offspring heterozygosity, conferring them survivorship and/or reproductive advantages in comparison to homozygotes (DeRose & Roff, 1999; Eizaguirre & Lenz, 2010). Mate preference for genetically dissimilar individuals (or avoidance of genetically similar individuals) has been shown in a wide range of species (Bretman, Newcombe, & Tregenza, 2009; Hoffman, Forcada, Trathan, & Amos, 2007; Kamiya, O'Dwyer, Westerdahl, Senior, & Nakagawa, 2014; Lovlie, Gillingham, Worley, Pizzari, & Richardson, 2013; Pizzari, Lovlie, & Cornwallis, 2004). Furthermore, given the selective advantages of heterozygosity, it has been proposed that individuals may also prefer more heterozygous mates (J. L. Brown, 1997), as found in several species (reviewed in Kempenaers, 2007). However, preference for heterozygous mates may be more likely to have evolved because they provide non-genetic benefits rather than genetic benefits, as heterozygosity might be heritable only under specific conditions (Fromhage, Kokko, & Reid, 2009; Kempenaers, 2007; Lehmann, Keller, & Kokko, 2007). Regardless of the preferred genetic characteristics, a preference generally implies that breeders have means to assess the genetic characteristics of their suitors.

*Genetic benefits and how to find them*

Under the “preference for good genes” hypothesis, suitors should invest in costly secondary sexual characters that are attractive for choosers because they honestly reflect their absolute

genetic quality (Jennions, Moller, & Petrie, 2001). Hamilton and Zuk (1982) proposed that expression of such characters depends on one's ability to fight parasites[2]. They found that the species of songbirds the most attacked by blood parasites also exhibited the most extravagant secondary sexual characters, and thus suggested that mate preference could be based on good genes for parasite resistance. In other words, only individuals that possess alleles conferring resistance against parasites would be in sufficient condition to afford high investment in secondary sexual characters. Expression of these attractive characters has thus to be costly to honestly reflect genetic quality (Zahavi, 1975). Expression of attractive characters may lead to reduced immunity, via immunosuppressive hormones or energy trade-off with other vital functions for instance, or to increased parasite exposure (Folstad & Karter, 1992; Sheldon & Verhulst, 1996; Zuk & Stoehr, 2002). Thus, individuals carrying good alleles for parasite resistance could deal with costs associated with strong expression of secondary sexual characters whereas those lacking these alleles would not be able to support reduced immunity or increased parasite exposure associated with expression of showy characters.

Regarding the cues used to assess compatible alleles, most studies have focused on odor cues, although there is also some rare evidence for the role of acoustic signals (McDonald & Wright, 2011). Assessment of genetic similarity over the whole genome or at specific loci through odor cues has been shown in species as diverse as humans (Wedekind, Seebeck, Bettens, & Paepke, 1995), sand lizards (*Lacerta agilis*) (Olsson et al., 2003), bank voles (*Myodes glareolus*) (Radwan, Tkacz, & Kloch, 2008), European storm petrels (*Hydrobates pelagicus*) (Bonadonna & Sanz-Aguilar, 2012; Leclaire, Strandh, Mardon, Westerdahl, &

[2] The term "parasite" refers here, and throughout the thesis, to all forms of infectious agents including bacteria, viruses, protozoa, fungi, helminth worms and arthropods

Bonadonna, 2017) and zebrafish (*Danio rerio*) (Gerlach & Lysiak, 2006). The mechanisms by which odors encode genetic similarity remain unclear but immune-related genes might play a role by shaping the community of bacteria responsible for the production of odors (Leclaire et al., 2019; D. J. Penn, 2002). Thus, as for good alleles, parasites are expected to play a role in the expression of characters permitting assessment of compatible alleles.

## MHC genes, parasites and fitness

The major histocompatibility complex (MHC or HLA in humans) is a cluster of genes coding for cell surface glycoproteins that are essential for the acquired immune system, and hence parasite resistance, making this gene family an excellent example of genetic benefits when studying sexual selection. MHC has been first described on mice by Georges Davis Snell in the 1940's but its functions and structure have been characterized in details more recently, mainly because of its pivotal role in human-related diseases. The role of MHC in parasite resistance and its high polymorphism have then led evolutionary biologists to study the selective pressures maintaining MHC-diversity and the selective advantages it confers in vertebrates. In this section, I give a brief description of the functions of MHC genes (Box 1), the hypotheses proposed to explain parasite-mediated selection on MHC genes, empirical evidence of MHC-parasite and MHC-fitness associations, and the factors these associations may depend on.

### Box 1 Function of the MHC

MHC genes encode cell-surface glycoproteins that bind peptide fragments coming from the degradation of foreign (parasite) or self-antigens in cells. After recognition of a peptide, an MHC molecule transports it on the cell surface for presentation to immune T-cells. If a T-cell

receptor recognize the MHC-peptide complex, an adaptive immune response is initiated (Murphy & Weaver, 2017). Because each MHC molecule can bind a restricted spectrum of peptides, the size of the MHC peptide-binding repertoire determines the number of different parasites against which the host is resistant.

There are two main classes of MHC molecules, known as class I and II (Murphy & Weaver, 2017). MHC class I molecules are located on the surface of all nucleated cells and present intracellular parasites (e.g. virus, some protozoa) to CD8+ cytotoxic T-cells (CTLs). CTLs proliferate, kill the infected cells and secrete cytokines that stimulate other immune cells, including macrophages that phagocyte parasites. In contrast, MHC class II molecules are only expressed on antigen-presenting immune cells (macrophages, dendritic cells, B-cells) and present extracellular parasites (e.g. bacteria, helminths) to naïve CD4+ T-cells. Activation of these naïve T-cells leads to their differentiation in different types of helper T-cells (Th1, Th2, Th17) that induce various immune responses, from the activation of macrophages to the production of antibodies.

MHC molecules can present both parasite-derived peptides and self-peptides, and have thus a central role in modulating self/non-self discrimination. T-cells recognize MHC-peptide complexes via a very specific receptor (TCR) which only binds to a specific combination of MHC molecule and peptide. The primary TCR diversity is generated by random nucleotide recombinations and rearrangements and is then shaped by two selection processes in the thymus. Positive selection retains only T-cells bearing a receptor capable to interact with any MHC-peptide complex, while negative selection eliminates T-cells carrying a receptor with too strong an affinity with MHC-self-peptide complexes, thereby preventing autoimmune reactions (L. Klein, Kyewski, Allen, & Hogquist, 2014).

Theoretical models have shown that high MHC-diversity should increase negative selection and thus decrease the TCR repertoire size, thereby limiting the potential for inducing immune reaction (Nowak, Tarczyhornoch, & Austyn, 1992). A recent study in bank voles has provided the first empirical evidence for this negative relationship between the TCR repertoire size and MHC class I, but not class II, diversity (Migalska, Sebastian, & Radwan, 2019). In contrast, other theoretical models have predicted that high MHC-diversity could enhance positive selection and thus lead to a relative enrichment of the TCR repertoire, including TCR with an affinity to self-antigens, thereby increasing the risk of autoimmune reactions (Borghans, Noest, & De Boer, 2003). Carrying many different MHC alleles may thus have a cost in terms of reduced efficiency of the immune response or increased risk of autoimmune reactions.

*MHC and parasite-mediated selection*

The MHC is the most polymorphic group of genes known in vertebrates and its role in parasite resistance has led to the hypothesis that parasite-mediated selection is the driving force maintaining this high level of genetic diversity. Three hypotheses have been proposed to explain how parasites can maintain MHC polymorphism.

**Rare allele advantage.** Because pathogens are more likely to adapt to the most common MHC alleles, new or rare MHC alleles should confer greater resistance against parasites until these alleles increase in frequency. Under this hypothesis, MHC polymorphism is thus maintained by negative frequency dependent selection from parasites, i.e. by a cyclical, co-evolutionary arm race between hosts and parasites in which MHC alleles and parasites change in frequency (Borghans, Beltman, & De Boer, 2004; Ejsmond & Radwan, 2015; Takahata & Nei, 1990).

**Fluctuating selection.** Because the composition of the parasite community fluctuates over time and space, different MHC alleles should be favored at different time and space. Under this

hypothesis, MHC polymorphism is maintained by directional, not cyclical, selection that fluctuates with environmentally driven change in parasite abundance and type (Hill et al., 1991).

**Heterozygote advantage (overdominance)**. Because each MHC protein can bind a limited set of antigens, carrying many different MHC alleles increases the number of antigens recognized, thereby providing resistance to a wider range of parasites. MHC heterozygotes, by carrying different MHC alleles, are thus expected to have increased resistance against a wider range of parasites compared to MHC homozygotes, leading to persistence of more MHC alleles in the population (Doherty & Zinkernagel, 1975; Hughes & Nei, 1988). Although the "heterozygote advantage" hypothesis has originally been tested in its literal sense, different aspects of MHC-diversity have been considered, such as the number of MHC alleles (across multiple loci) carried by an individual (Bentkowski & Radwan, 2019) or the functional divergence between the MHC alleles carried by an individual (Wakeland et al., 1990). The latter, termed as the **divergent allele advantage** hypothesis, considered the antigen binding properties of MHC alleles and proposed that individuals carrying alleles with dissimilar antigen binding properties should present a wider range of antigens to immune cells, thereby allowing recognition of a wider range of parasites in comparison to individuals carrying functionally similar alleles (Pierini & Lenz, 2018). Finally, the paradox that the number of different MHC alleles found within an individual is generally much lower than the number of different alleles found in a population, has led researchers to propose that there must be costs associated with too high MHC-diversity. Although these costs remain poorly known (Box 1), there is theoretical and empirical support that parasite resistance is maximized at intermediate, not maximal, MHC-diversity (Kloch, Babik, Bajer, Sinski, & Radwan, 2010; Madsen & Ujvari, 2006; Nowak et al., 1992; Wegner, Kalbe, Kurtz, Reusch, & Milinski, 2003).

*Empirical evidence for MHC-parasite associations*

Although disentangling these different hypotheses is proved to be a difficult task (Spurgin & Richardson, 2010), the numerous studies that investigated MHC-parasite associations in a wide range of non-model species have provided valuable insights into the genetic bases of parasite resistance (reviewed in Sin et al., 2014; see also Hacking, Stuart-Fox, Godfrey, & Gardner, 2018; Sepil, Lachish, Hinks, & Sheldon, 2013). Many studies have reported associations between specific MHC alleles and measures of parasite resistance while there is less support for an advantage of MHC-diversity (reviewed in Sin et al., 2014). Because MHC-diverse individuals may be advantaged only when confronted to multiple parasites (McClelland, Penn, & Potts, 2003; D. J. Penn, Damjanovich, & Potts, 2002), the relatively low support for an advantage of MHC-diversity may be due to the fact that many studies have been conducted in captive or semi-natural conditions. These environments might be more benign, and thus less challenging for individuals, because they comprise a less diverse parasitic fauna than natural populations.

Although several studies in natural populations have reported associations between MHC and parasite prevalence (i.e. infected or not), there is generally no clear understanding of how MHC confer resistance against parasites. Because individuals are generally not sampled multiple times over time, it is often difficult to distinguish between qualitative resistance (i.e. prevention of parasite infection), quantitative resistance (i.e. limitation of parasite infection) or susceptibility (i.e. increased risk of infection) (Westerdahl, Asghar, Hasselquist, & Bensch, 2012). For instance, the direction of the association between an MHC allele conferring quantitative resistance and parasite prevalence might depend on the timing of sampling, i.e. it may be positive if individuals have just been infected or it may be negative if individuals have had sufficient time to clear infection. Additionally, a positive association between an MHC

allele and parasite prevalence can indicate increased susceptibility or, alternatively, limitation of the development of the infection without total clearance (Westerdahl et al., 2012). One possible solution to disentangle these different mechanisms is to monitor parasite infection intensity (i.e. how severe is the infection) in addition to parasite prevalence (Westerdahl et al., 2012). However, parasite infection intensity might still depend on the timing of sampling, making the longitudinal monitoring of parasite infection the best option when possible. To my knowledge, no study has linked MHC to parasite infection by following the course of the infection in natural populations.

*Empirical MHC- fitness associations*

Fitness advantages of MHC are generally inferred from MHC-parasite associations but this assumption is generally not confirmed. Yet, some studies have directly investigated associations between MHC and proxies of fitness in semi-natural and natural populations. Certain MHC alleles have been associated with fitness advantages, in terms of reproductive success or survival (Brouwer et al., 2010; de Assuncao-Franco, Hoffman, Harwood, & Amos, 2012; Osborne et al., 2015; Paterson, Wilson, & Pemberton, 1998; Sepil, Lachish, & Sheldon, 2013). Additionally, several studies have reported associations between MHC-diversity and fitness, with fitness advantages being found at either maximal (Brouwer et al., 2010; de Assuncao-Franco et al., 2012; Dunn, Bollmer, Freeman-Gallant, & Whittingham, 2013; Lenz, Mueller, Trillmich, & Wolf, 2013; Osborne et al., 2015) or intermediate MHC-diversity (Kalbe et al., 2009; Madsen & Ujvari, 2006; Wegner, Kalbe, et al., 2003). Other studies found no fitness advantage of MHC-diversity or specific MHC alleles (Karlsson et al., 2015; Radwan et al., 2012). One reason for these mixed results may be that survival is generally not monitored in early-life stages even though this life stage is often characterized by an elevated mortality rate (Hemmings, Slate, & Birkhead, 2012; Low & Part, 2009; Sullivan, 1989), which sometimes

results from parasite infections (Benskin, Wilson, Jones, & Hartley, 2009). A handful of studies investigating MHC-survival associations have considered juvenile mortality before independence (Karlsson et al., 2015; Lenz et al., 2013; Osborne et al., 2015). Ignoring early-life stages may yield only a partial picture of how MHC affects survival, and fitness more generally, if selection removes the less fit genotypes early in life, leaving only relatively high-quality individuals that survived long enough to be sampled.

*All in the same boat? Factors modulating MHC-fitness associations*

Another reason for the mixed results obtained regarding the association between MHC-diversity and fitness may be that the vast majority of studies considered that, within a population, all individuals should benefit from MHC-diversity the same way. However, how MHC-diversity affects fitness certainly depends on the costs and benefits associated with MHC-diversity (Box 1) and should therefore depend on any trait expected to modulate this cost-benefit balance, such as exposure to parasites, immune response strength, or susceptibility to autoimmune disorders. Most studies that investigated associations between MHC-diversity and fitness considered all individuals as being equally exposed to parasites or equally capable of mounting an immune response. Yet, there is extensive evidence for interindividual variations in exposure and immune responses to parasites (Barber & Dingemanse, 2010; Roved, Westerdahl, & Hasselquist, 2017; Tompkins, Dunn, Smith, & Telfer, 2011; van der Most, de Jong, Parmentier, & Verhulst, 2011). A handful of studies have explored interindividual variation in the optimal level of MHC-diversity (i.e. the level of diversity at which fitness is maximized) by investigating whether it depends on sex. These studies showed positive associations between MHC-diversity and survival or reproductive success in adult males, while results were more equivocal in adult females, with either positive, negative or no association being reported (Huchard, Knapp, Wang, Raymond, & Cowlishaw, 2010; Roved, Hansson,

Tarka, Hasselquist, & Westerdahl, 2018; Sauermann et al., 2001; Schaschl et al., 2012). As these studies were restricted to polygynous species, positive associations between MHC-diversity and fitness in adult males were explained by their increased risk of wounds and thus infections during breeding and/or by the immunosuppressive effects of testosterone. However, whether optimal MHC-diversity varies with sex in species with different life-history strategies and whether it varies with other traits than sex remains to be investigated.

## MHC genes and reproductive strategies

In addition to be maintained by selection from parasites, MHC polymorphism has been suggested to be mediated by sexual selection (Ejsmond, Radwan, & Wilson, 2014; Hedrick, 1992; Milinski, 2006). The first evidence for a role of MHC genes in sexual selection came from studies in laboratory mice suggesting odor-based recognition of MHC genes and the use of this cue in mating decisions (Yamazaki et al., 1976; Yamazaki et al., 1979). Numerous studies have then continued to investigate the role of MHC genes in reproductive strategies, a vast majority of which concerns precopulatory mate choice.

*MHC-based mate choice*

Several mechanisms for the role of MHC genes in mate choice have been proposed (reviewed in Kamiya et al., 2014). A first hypothesis states that individuals may be preferred because they carry "good" MHC alleles (i.e. rare or new MHC alleles) or combinations of MHC alleles that confer a selective advantage to offspring through increased resistance against parasites. Acquisition of genetic benefits via the transfer of specific MHC alleles may also be achieved by reproducing with individuals carrying many different MHC alleles (i.e. more MHC-diverse or heterozygous individuals). This is because "good" MHC alleles are expected to be rare in a

population and thus to occur disproportionately in heterozygotes (Apanius, Penn, Slev, Ruff, & Potts, 1997). Reproducing with an MHC-diverse individual has also been proposed to be beneficial because it may result in higher MHC-diversity in offspring (Kamiya et al., 2014). However, although mate choice for heterozygosity across many loci (i.e. genome-wide) may evolve under certain conditions in absence of direct, non-genetic, benefits, this should not be the case when selection targets a few loci (e.g. the MHC in most species) (Fromhage et al., 2009; Lehmann et al., 2007). Nonetheless, specific MHC alleles and MHC-diversity have been associated with the expression of characters (e.g. ornaments, displays) that choosers could use to evaluate the genetic quality of their suitors (Huchard, Raymond, et al., 2010; Slade, Watson, & MacDougall-Shackleton, 2017; von Schantz, Wittzell, Goransson, Grahn, & Persson, 1996; Whittingham, Freeman-Gallant, Taff, & Dunn, 2015). Accordingly, increased reproductive success has been associated with high MHC-diversity or with specific MHC alleles (Bonneaud, Chastel, Federici, Westerdahl, & Sorci, 2006; Eizaguirre et al., 2009; Ekblom et al., 2004; Hoover et al., 2018; Sauermann et al., 2001), suggesting reproductive preferences for MHC-diverse individuals or for those carrying specific MHC alleles. It should be noted that individuals carrying "good" MHC alleles, many different or divergent MHC alleles are expected to have increased health and condition because of their increased resistance against parasites, thereby allowing them to provide increased direct benefits to choosers (e.g. increased food-provisioning, better-quality territory, no parasite contamination). Thus, these individuals may be more likely to be preferred because they provide direct benefits rather than genetic benefits in resource-based mating systems where direct benefits are important (J. Winternitz, Abbate, Huchard, Havlicek, & Garamszegi, 2017).

Another, non-exclusive, MHC-based mate choice hypothesis posits that individuals may be preferred because they carry MHC alleles that are "compatible" to the chooser's own MHC

alleles. MHC compatible individuals are not preferred for their intrinsic genetic quality and the associated direct benefits, but for the acquisition of genetic benefits through optimization of offspring MHC-diversity (Zeh & Zeh, 1996, 1997). By reproducing with MHC-dissimilar mates, an individual increases MHC-diversity in the progeny, thereby providing to offspring an increased protection against parasites. MHC-dissimilar parents may also provide to offspring a "moving target" against parasite adaptation to MHC. By being MHC-dissimilar to both parents, offspring may be protected against rapidly evolving parasites that have escaped parental MHC-mediated immune recognition (D. J. Penn & Potts, 1999). Precopulatory preferences for MHC-dissimilar mates have been experimentally demonstrated in model species such as mice (reviewed in D. J. Penn & Potts, 1999). Other studies have found a preference for the odor of MHC-dissimilar individuals in mice and humans by using experimental choice tests where animals were given a choice between odor samples from different MHC genotypes (Ninomiya & Brown, 1995; Wedekind & Furi, 1997; Wedekind et al., 1995). These investigations have then been extended to wild animals and preference for MHC-dissimilar individuals has been shown by conducting choice test experiments in controlled conditions (Bahr, Sommer, Mattle, & Wilson, 2012; Leclaire et al., 2017; Olsson et al., 2003; Radwan et al., 2008) or by testing whether observed MHC-distance between mates is on average higher than what would be expected if mating is random with respect to MHC-distance (Huchard, Baniel, Schliehe-Diecks, & Kappeler, 2013; Santos, Michler, & Sommer, 2017; Strandh et al., 2012).

Alternative versions of this MHC compatible hypothesis have been proposed. First, if parasite resistance is maximized at intermediate MHC-diversity rather than at maximal MHC-diversity (Milinski, 2006; Nowak et al., 1992), then individuals should prefer suitors harboring an intermediate MHC-distance to produce offspring with an intermediate MHC-diversity. Evidence for this intermediate MHC-distance preference mainly comes from studies in three-

spined sticklebacks (*Gasterosteus aculeatus*) (Aeschlimann, Haberli, Reusch, Boehm, & Milinski, 2003; Milinski et al., 2005; Reusch, Haberli, Aeschlimann, & Milinski, 2001), in which parasite resistance and fitness are maximized at an intermediate level of MHC-diversity (Kalbe et al., 2009; Wegner, Kalbe, et al., 2003). Second, in addition to increased offspring resistance against parasites, a preference for MHC-dissimilar individuals may have evolved to avoid inbreeding (i.e. the mating of genetically related individuals) (Ruff, Nelson, Kubinak, & Potts, 2012). As numerous studies have reported a negative effect of inbreeding on fitness (DeRose & Roff, 1999, Keller & Waller, 2002, Charlesworth & Charlesworth, 1987), especially in early-life stages (Hemmings et al., 2012), a preference for MHC-dissimilar individuals may decrease the fitness costs of inbreeding depression by increasing genome-wide heterozygosity in the progeny. While some studies found a positive correlation between MHC-diversity and genome-wide heterozygosity, others did not, suggesting that MHC-linked inbreeding avoidance may exist under specific conditions (Ruff et al., 2012).

*MHC and postcopulatory strategies*

Because sexual selection can continue after mating, researchers have suggested that postcopulatory strategies may depend on MHC genes (Wedekind, 1994; Ziegler, Kentenich, & Uchanska-Ziegier, 2005). There is few evidence of a fertilization advantage for MHC-diverse males or for those carrying specific alleles (Skarstein, Folstad, Liljedal, & Grahn, 2005) while several studies found a fertilization bias according to MHC-compatibility between mates. Egg-fertilization bias toward sperm from MHC-dissimilar males has been shown in the red junglefowl (*Gallus gallus*) (Lovlie et al., 2013) whereas sperm from MHC-similar males was advantaged in other species (C. Gasparini, Congiu, & Pilastro, 2015; Geßner, Nakagawa, Zavodna, & Gemmell, 2017; Yeates et al., 2009). These fertilization biases suggest cryptic female preference for the sperm of males with particular MHC characteristics, possibly via

selective effects of ovarian fluids on sperm or via non-random fusion of the gametes (C. Gasparini & Pilastro, 2011; Rulicke, Chapuisat, Homberger, Macas, & Wedekind, 1998; Wedekind, Chapuisat, Macas, & Rulicke, 1996). Moreover, there is evidence that males also may bias fertilization success according to the MHC of females. In particular, males have been found to invest more into ejaculate quality (e.g. sperm number, sperm viability) when in presence of MHC-dissimilar females (Burger, Dolivo, Marti, Sieme, & Wedekind, 2015; Gillingham et al., 2009; Jeannerat et al., 2018). It remains unknown whether such male strategies increase their fertilization success and/or provide advantages to the developing embryo, as sperm quality might have long-term fitness consequences on offspring quality (Immler, Hotzy, Alavioon, Petersson, & Arnqvist, 2014).

*MHC and post-fertilization reproductive strategies*

Although MHC-based reproductive strategies have been found in a wide range of species, it is worth noting that many studies did not find any role of MHC genes in reproductive decisions (Huchard, Knapp, et al., 2010; Kuduk et al., 2014; Paterson & Pemberton, 1997; Sepil et al., 2015; Westerdahl, 2004). Because sexual selection might act continuously on MHC genes from the level of mating to the level of offspring production, MHC-linked preferences could exist in reproductive decisions that were not investigated in these studies.

It is largely unknown whether MHC genes play a role in reproductive decisions after fertilization, i.e. whether parents improve genetic quality of their progeny through differential investment among offspring (Burley, 1986, 1988; Sheldon, 2000). MHC-based differential investment in offspring after fertilization may have evolved to compensate or accentuate pre-fertilization reproductive decisions. Such strategies may be particularly expected when individuals face constraints in choosing within a limited pool of potential mates and when they

reproduce with one mate only, i.e. when the opportunity for pre- and/or postcopulatory strategies is limited. Even if MHC-based post-fertilization strategies may exist in all mating systems, the rarity of genetically monogamous species may explain why such strategies have not received much attention in the context of MHC-linked reproductive strategies.

Yet, studies in laboratory mice and humans have explored whether females selectively abort offspring to adjust their investment in the progeny according to the male's MHC. In mice, females are more likely to terminate pregnancy when exposed after mating to the odor from a new male that is MHC-dissimilar to the progenitor (Yamazaki et al., 1983). Such strategy, called the "Bruce effect", is adaptive for females because they avoid the costs of pregnancy if their offspring are likely to be killed by a new male (Bruce, 1959). Spontaneous abortion has also been linked to the progenitor's MHC in humans, with an increased risk of abortion found when couples are relatively MHC-similar (C. Ober, Elias, Kostyu, & Hauck, 1992; but see Meuleman et al., 2015). Moreover, these MHC-linked abortions have been suggested to be sex-specific. Newborn males have been found to be more MHC-diverse compared to newborn females in mice and humans (Dorak, Lawson, Machulla, Mills, & Burnett, 2002 and references therein), suggesting increased mortality of low MHC-diverse sons. While this bias may result from a cryptic female choice according to the father's MHC, it may also be explained by a lethal incompatibility between parents' genotypes. Interestingly, a recent study in horses (*Equus caballus*) performed artificial inseminations to exclude the genetic incompatibility explanation (Burger et al., 2017). They found a decrease in early pregnancy failures when females were exposed to an MHC-dissimilar male in comparison to when they were exposed to an MHC-similar male, while the MHC of the sperm donor (a different male) had no effect on pregnancy failure. Although the sex of the embryos was not known, this study suggests that females reject embryos according to the male's MHC.

Sex allocation theory predicts that parents should adjust their investment in daughters and sons depending on the fitness costs and benefits associated with each sex (Charnov, 1982; Frank, 1990; Trivers & Willard, 1973). Sex-ratio adjustment can depend on the mate's genetic quality because the transfer of its genes might differently affect the viability or reproductive success of sons and daughters (Booksmythe, Mautz, Davis, Nakagawa, & Jennions, 2017; Burley, 1981; West, 2009, chapter 6). While most empirical studies have considered the absolute mate quality, the relative fitness of daughters and sons can also depend on the genetic compatibility between mates (Brekke, Bennett, Wang, Pettorelli, & Ewen, 2010; Pryke & Griffith, 2009b; Rioux-Paquette, Festa-Bianchet, & Coltman, 2011). Accordingly, a sex ratio adjustment has been associated with pair relatedness in lance-tailed manakins (*Chiroxiphia lanceolata*) (Sardell & DuVal, 2014) and with a genetic incompatibility at a Z-linked gene in Gouldian finches (*Erythrura gouldiae*), (Pryke & Griffith, 2009a). However, it remains unknown whether sex ratio adjustment depends on MHC-similarity between mates. If low MHC-diversity is more detrimental to one sex than to the other, then we should expect MHC-similar parents to be selected to overproduce the sex that suffer the least from low MHC-diversity.

## Aims of the thesis

In this thesis, I investigated the selective advantages of certain genetic characteristics and I explored whether individuals can adjust their reproductive decisions depending on the prospective genetic characteristics of their offspring. Much of the work involves MHC class II genes and long-term breeding data on a population of the monogamous seabird black-legged kittiwake (*Rissa tridactyla*). This thesis consists in three chapters and has five specific goals:

i. Early-life stages are characterized by a relatively high mortality rate in numerous species, including in some populations of black-legged kittiwakes (Barrett & Runde, 1980; Gill, Hatch, & Lanctot, 2002) Parasites, in particular ticks, have been suggested to have important non-lethal and lethal effects in kittiwake chicks (Chastel, Monnat, Lelay, & Balouet, 1987; McCoy, Boulinier, Schjorring, & Michalakis, 2002). Because MHC class II molecules can recognize antigens contained in tick saliva and present them to T cells (Andrade, Texeira, Barral, & Barral-Netto, 2005), the MHC class II is well indicated to play a role in tick resistance in kittiwakes, as found in mammals and reptiles (Hacking et al., 2018; Oliver, Telfer, & Piertney, 2009). A first goal was to investigate associations between functional MHC (diversity, specific alleles) and tick resistance in kittiwake chicks by sampling them multiple times to follow thoroughly the infection (**Chapter 1**).

ii. Although MHC-parasite associations are important to have a better understanding of the genetic bases of host-parasite resistance, they do not allow direct inference of how MHC genes explain fitness differences between individuals in wild populations. A second goal was to associate functional MHC to two other important fitness-related traits in addition to tick infection, namely growth and survival before independence (**Chapter 1**).

iii. A vast majority of MHC-fitness studies have considered that, within a population, all individuals should benefit from MHC-diversity the same way, i.e. that all individuals are equally exposed to parasites or equally capable of mounting an immune response. This is certainly not realistic in wild populations. A third, major goal was to test whether the optimal level of MHC-diversity varies with sex and hatching order, two traits expected to modulate exposure and immune response to parasites in kittiwake chicks (**Chapter 1**).

iv. The sex-specific associations we found between MHC-diversity and fitness-related traits led us to put forward that parents should adjust sex ratio in their progeny according to their MHC-similarity. A fourth goal was to investigate whether MHC-similar parents adaptively overproduce sons to avoid production of low MHC-diverse, less fit daughters (**Chapter 2**).

v. In addition to be associated with parasites, breeding failure in kittiwakes have been suggested to depend on inbreeding (Mulard et al., 2009) and sperm ageing (i.e. the post-meiotic senescence of sperm cells; Wagner, Helfenstein, & Danchin, 2004; White et al., 2008). These reproductive costs might be limited, respectively, by kittiwakes preferentially pairing with genetically-dissimilar mates and by females preferentially ejecting sperm following precocious copulations (i.e. sperm that would have been old by the time of fertilization; Mulard et al., 2009; Wagner et al., 2004). However, the high variance observed in these behaviors suggests that not all individuals pair with a genetically dissimilar mate and that other factors than sperm ageing modulate sperm ejections. If sperm ageing exacerbates the detrimental effects of inbreeding, individuals paired with genetically-similar mates may preferentially avoid fertilization by old sperm. A fifth goal was to investigate whether an interplay between inbreeding and sperm ageing modulates fitness-related traits and whether the behavioral strategies preventing fertilization by old sperm depend on genetic similarity between mates (**Chapter 3**).

## Study species and site

The black-legged kittiwake is a pelagic gull species (Laridae) commonly found in the northern hemisphere, from Alaska to northern Europe. Kittiwakes breed each year (April-August) in

dense colonies on sea cliffs, usually after four years old and over twenty years (Hatch, Robertson, & Baird, 2009). They are strictly monogamous, being faithful to one partner during the breeding season (Helfenstein, Tirard, Danchin, & Wagner, 2004) and generally retaining the same partner over several years, although divorce can occur after breeding failure (Naves, Cam, & Monnat, 2007). Parents cooperate and equally share tasks during the breeding season, from nest construction to food provisioning during the chick-rearing period (J. C. Coulson & Wooller, 1984; Cullen, 1957; Roberts & Hatch, 1993). The typical clutch size is two eggs (range 1-3; J. C. Coulson & White, 1958), with a 27-days long incubation period after which semi-precocial chicks hatch (eyes open, covered with down, mobile) and develop at the nest until they reach adult size and fledge (i.e. 40 days; Hatch et al., 2009). Parents and fledglings leave the colonies at the end of the breeding season and spend winter in the northern Atlantic and Pacific oceans.

The vast majority of the data used in this thesis has been collected on a population of kittiwakes breeding on Middleton Island (59°26'N, 146°20'W), Gulf of Alaska (Photo 1A). Kittiwakes mostly nest on abandoned buildings dating from the 1950s when the U.S. Air Force was on the island (Photo 1B). In particular, the upper walls of an abandoned radar tower have been converted into artificial cliffs by Scott A. Hatch in the 1990s (Photo 1C, D). One-way mirrors allow close monitoring of the natural behavior of more than 300 kittiwake pairs from inside the tower (Photo 1E-J). Eggs and chicks can be captured throughout the breeding season by sliding the mirrors, and a small gap below the mirrors allow the capture of adults using a hook (Gill & Hatch, 2002). Behavioral observations used in Chapter 3 were collected on a kittiwake population nesting on sea cliffs at Cap Sizun in Brittany, France (48°5'N, 4°36'W) (Cam, Hines, Monnat, Nichols, & Danchin, 1998). In both populations, adults and chicks are identified individually using a combination of rings (color and/or metal).

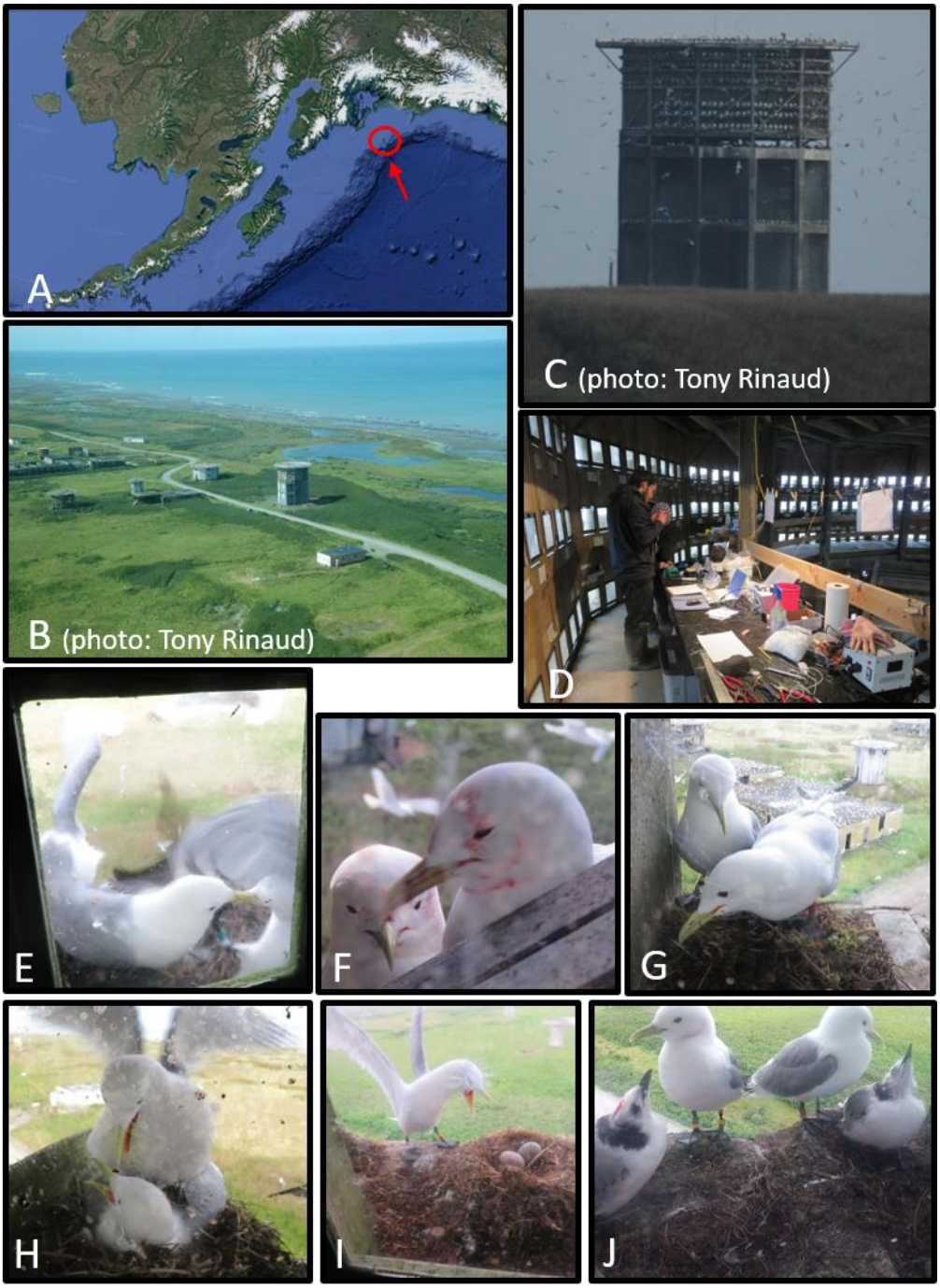


**Photo 1. A:** Location of Middleton Island in the Gulf of Alaska. **B:** Aerial view of Middleton Island, with abandoned U.S. Air Force buildings. **C:** Closer view of the abandoned radar tower converted into a kittiwake observatory. **D:** Inside view of the abandoned radar tower. Each window is a one-way mirror overlooking a nest site. **E:** Adult kittiwakes fighting for a nest site. **F:** A kittiwake pair after a fight for a nest site. **G:** A kittiwake pair building its nest. **H:** A kittiwake pair copulating. **I:** An adult kittiwake and its two eggs. **J:** Two adult kittiwakes and their begging chicks.

# CHAPTER 1: Sex and hatching order modulate the association between MHC-II diversity and fitness in early-life stages of a wild seabird

Molecular Ecology, accepted

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## ABSTRACT

Genes of the major histocompatibility complex (MHC) play a pivotal role in parasite resistance, and their allelic diversity has been associated with fitness variations in several taxa. However, studies report inconsistencies in the direction of this association, with either positive, quadratic or no association being described. These discrepancies may arise because the fitness costs and benefits of MHC-diversity differ among individuals depending on their exposure and immune responses to parasites. Here, we investigated in black-legged kittiwake (*Rissa tridactyla*) chicks whether associations between MHC class-II diversity and fitness vary with sex and hatching order. MHC-II diversity was positively associated with growth and tick loss in female chicks, but not in male chicks. Our data also revealed a positive association between MHC-II diversity and survival in second-hatched female chicks (two eggs being the typical clutch size). These findings may result from condition-dependent parasite infections differentially impacting sexes in relation to hatching order. We thus suggest that it may be important to account for individual heterogeneities in traits that potentially exert selective pressures on MHC-diversity in order to properly predict MHC-fitness associations.

*Keywords:* Divergent allele advantage; fitness; heterozygote advantage; immunity; Ixodes uriae; parasite-mediated selection

## INTRODUCTION

Identifying the genetic bases of fitness differences among individuals is a long-standing goal in evolutionary biology (Chapman, Nakagawa, Coltman, Slate, & Sheldon, 2009; Ellegren & Sheldon, 2008; Merila & Sheldon, 1999). In this context, immune-related genes have drawn substantial attention (Bateson et al., 2016; Froeschke & Sommer, 2005; Oliver et al., 2009; Sepil, Lachish, Hinks, et al., 2013) because parasite resistance often covaries with fitness in

wild populations (Asghar et al., 2015; Hamilton & Zuk, 1982; Moller, Arriero, Lobato, & Merino, 2009; Willink & Svensson, 2017). The major histocompatibility complex (MHC) is a cluster of genes coding for cell surface proteins that are essential for the adaptive immune system. The MHC plays a critical role in modulating self/non-self discrimination and in activating immune response against parasites (Murphy & Weaver, 2017). In past decades, several studies reported associations between MHC allelic diversity and fitness, but with some major inconsistencies. For instance, depending on the considered species or population, either maximal or intermediate MHC-diversity was found to maximize fitness (Bonneaud, Mazuc, Chastel, Westerdahl, & Sorci, 2004; Kalbe et al., 2009; Lenz et al., 2013; Thoss, Ilmonen, Musolf, & Penn, 2011; Wegner, Reusch, & Kalbe, 2003).

The reported inconsistencies in the fitness consequences of MHC-diversity likely stem from the variations in the trade-off between the different functions of the MHC. The fact that each MHC protein can bind a limited set of antigens leads to the straightforward expectation that an increase in MHC diversity increases the number of antigens recognized, thereby providing resistance to a wider range of parasites (reviewed in Milinski, 2006). Higher MHC-diversity may also lead to more efficient immune responses during infection (Behnke & Wahid, 1991; Doherty & Zinkernagel, 1975; McClelland et al., 2003). However, theoretical models have shown that high MHC-diversity can also incur costs by limiting the potential for inducing an immune reaction (Nowak et al., 1992; Woelfing, Traulsen, Milinski, & Boehm, 2009; see also Migalska et al. 2019) or by increasing the potential for autoimmune disorders (Borghans & De Boer, 2001).

Variation in the level of MHC-diversity maximizing fitness should be shaped by any selective pressure associated with changes in exposure to parasites, immune response strength or susceptibility to autoimmunity. Several studies have shown that MHC-diversity varies

among species or populations according to key life-history traits. For instance, increased mean MHC-diversity has been associated with migratory behavior and female promiscuity, two traits that may increase exposure and/or reduce immune response to parasites (Gohli et al., 2013; Minias, Pikus, Whittingham, & Dunn, 2019; Whittingham, Dunn, Freeman-Gallant, Taff, & Johnson, 2018; J. C. Winternitz et al., 2013). In contrast, studies investigating interindividual variation in the fitness consequences of MHC-diversity within a population are scarce, although there is extensive evidence for interindividual variations in these potential selective pressures (i.e. exposure to parasites, immune response strength or susceptibility to autoimmunity). For instance, it has been recently hypothesized that sex differences in the effects of immunosuppressive sex hormones on the strength of immune response and on the susceptibility to autoimmunity should result in different optima of MHC diversity between males and females (Roved et al., 2017). Specifically, the authors argued that males should benefit from higher levels of MHC-diversity than females because sex hormones reduce immune activation in males and increase susceptibility to autoimmunity in females. Accordingly, the association between MHC-diversity and reproductive success was positive in adult males but not in adult females in great reed warblers (*Acrocephalus arundinaceus*) (Roved et al., 2018). This hypothesis may explain similar findings in other species (Huchard, Knapp, et al., 2010; Sauermann et al., 2001; Schaschl et al., 2012). There may be other explanations for why males benefit from higher levels of MHC-diversity than females. For instance, male-male contests increase males' risk of wounds and thus infections (Huchard, Knapp, et al., 2010 and references therein), and deplete male's energetic reserves, thereby possibly leading to less energy available for allocation to immune functions (Schaschl et al., 2012 and references therein). While a few other studies investigated sex-specific associations between MHC-diversity and fitness (Hablutzel et al., 2014; Jager et al., 2007; Lenz, Eizaguirre, Scharsack, Kalbe, & Milinski,

2009), no studies have investigated whether other individual traits may modulate these associations. Sex is not the only trait modulating exposure to parasites or immune response strength. For instance, social status and personalities have been associated with infection risks by influencing frequency and duration of interactions with conspecifics (Boyer, Reale, Marmet, Pisanu, & Chapuis, 2010; Drewe, 2010; Habig & Archie, 2015).

Here, we investigated whether differences in sex and hatching order are associated with variation in the fitness consequences of MHC class-II diversity during the nestling stage in a wild population of the monogamous black-legged kittiwakes (*Rissa tridactyla*). In kittiwakes, female and second-hatched chicks are smaller, grow slower (T. Merkling et al., 2012; Vincenzi, Hatch, Mangel, & Kitaysky, 2013; Vincenzi, Hatch, Merkling, & Kitaysky, 2015) and suffer more from sibling aggressions (Delaunay, 2018; White, Leclaire, et al., 2010) than other chicks, suggesting that they are less competitive for food and in poorer condition. In several other species, including birds, food shortage and reduced condition have been linked to reduced immune responses via energy trade-offs (Beldomenico & Begon, 2010; Brzek & Konarzewski, 2007) or chronic stress (Glaser & Kiecolt-Glaser, 2005). In addition, in kittiwakes, second-hatched chicks hatch from eggs containing increased levels of androgens compared to first-hatched chicks (Benowitz-Fredericks, Kitaysky, Welcker, & Hatch, 2013; J. Gasparini et al., 2007). This may lead to reduced immune responses as these sex hormones are known to be immunosuppressive in other species (S. L. Klein & Flanagan, 2016; Smyth, Caruso, Davies, Clutton-Brock, & Drea, 2018). Thus, females and second-hatched chicks are predicted to be immunologically disadvantaged compared to males and first-hatched chicks. Because, individuals who have less efficient immune response are hypothesized to be particularly advantaged by high MHC-diversity (Roved et al., 2017), we expect, the fitness of females and second-hatched chicks to be more dependent on MHC-diversity than the fitness of males and

first-hatched chicks. We thus investigated whether the association between fitness-related traits and MHC class-II diversity varied with sex and hatching order. We tested several fitness-related traits, namely survival in the nest, growth rate and tick infection during the nestling stage. Growth rate is an important component of fitness in kittiwakes because faster growing chicks are more likely to recruit as breeders (Vincenzi et al., 2015). Ticks can have strong deleterious effects on kittiwake chicks by reducing growth rate when food is scarce (McCoy et al., 2002), potentially leading to death in case of hyper-infestation (Chastel et al., 1987). Antigens contained in tick saliva are recognized by MHC class II molecules, which present them to T lymphocytes, thereby activating an immune response that can reduce tick-feeding efficiency and eventually lead to tick detachment (Andrade et al., 2005; Oliver et al., 2009; Owen, Nelson, & Clayton, 2010).

## MATERIALS AND METHODS

### Study site

The study was conducted during the 2007-2013 and 2016-2017 breeding seasons (May–August) on a colony of black-legged kittiwakes nesting on an abandoned U.S. Air Force radar tower on Middleton Island (59°26'N, 146°20'W), Gulf of Alaska. The 400 nest sites created on the upper walls of the tower can be observed from inside the building through sliding one-way mirrors and birds are individually identified using color and metal bands (Gill & Hatch, 2002). All nest sites were checked twice daily (9:00 and 18:00) to record laying, hatching and death events. We focused on two-eggs clutches, which is the typical clutch size in this kittiwake population (range 1-3; Gill & Hatch, 2002). On the day of laying, A- and B- eggs (first- and second-laid eggs, respectively) were labeled individually with a non-toxic marker. Chicks were marked on the head with similar markers for identification shortly after hatching.

## DNA collection and sexing

Chicks were sexed molecularly using DNA extracted from eggshells, tissues from embryos (in case of pre-hatching death) or blood collected from the metatarsal vein (see T. Merkling et al., 2012 for a detailed sexing protocol). A few chicks (n = 18) were sexed *a posteriori* when they came back to the colony as adults (identified with a numbered metal ring). We used DNA extracted from a blood sample collected on adults with a syringe or capillaries from the brachial vein to determine sex using the same molecular method as for chicks (T. Merkling et al., 2012). The sex of a few adults (n = 4) was determined using sex-specific behavior (i.e. copulation and courtship feeding during the prelaying period; E. Danchin, 1991; Jodice, Lanctot, Gill, Roby, & Hatch, 2000).

## Molecular analysis of major histocompatibility complex

### *MHC genotyping*

The DNA samples were used to amplify 258 bp fragments (218 bp excluding primers) of the exon 2 of the black-legged kittiwake MHC class-IIB. We used the MHC class-IIB specific primers (forward: 5' GCACGAGCAGGGTATTTCCA and reverse: 5' GTTCTGCCACACACTCACC) designed by Leclaire et al. (2014), which amplify at least two MHC class-IIB loci. These loci are presumed to be functional as shown by signs of positive selection, by the absence of stop codon or frame shift mutations in the translated alleles and by at least three alleles being transcribed in each of the two individuals studied (Leclaire et al., 2014). To discriminate samples after sequencing, the 5′ end of both forward and reverse primers included a combination of two different 8 bp tags. The PCR amplification was performed in 20 µl mixtures containing 2 µl of extracted DNA, 0.5 µM of each primer, 1x AmpliTaq Gold® 360 Master Mix (Applied Biosystems, Foster City (CA) USA) and 3.2 µg of bovine serum

albumin (Roche Diagnostics, Basel, Switzerland). The PCR program consisted of 10 min initial denaturation at 95°C, followed by 30 cycles of 30 s denaturation at 95°C, 30 s annealing at 57°C and 1 min 30 s extension at 72°C. A final elongation step was run at 72°C for 7 min. Amplicons were then purified using the MinElute PCR Purification protocol (Qiagen, Hilden, Germany). Amplicons were sequenced in two runs with an Illumina MiSeq platform, using the 2 × 300 bp protocol (Fasteris SA, Plan-les-Ouates, Switzerland). We included PCR blank controls, as well as unused tag combinations, in the sequenced multiplex to detect and withdraw potential mistagging biases (Esling, Lejzerowicz, & Pawlowski, 2015).

Amplicon sequences were analyzed with ampliSAS, a three-step pipeline that consists of read demultiplexing, unique sequence clustering, and erroneous sequence filtering (Sebastian, Herdegen, Migalska, & Radwan, 2016). First, the Illumina data were filtered to remove low-quality sequences (sequences with Phred scores less than 50). Sequences were then clustered using the default ampliSAS parameters for Illumina sequences (substitution errors: 1%, indel errors: 0.001%, minimum frequency with respect to dominant: 25%), and sequences that were potential chimeras or that had less than 3% frequency were discarded. We discarded samples with a depth inferior to 500 reads. Considering the larger set of samples analyzed in the two MiSeq runs (n = 2064 samples, including 699 samples that were part of this study), the reproducibility of genotype between the two runs (n = 25 DNA samples that were split and processed in independent PCRs) was 100%. After processing, we had an average (± s.d.) of 4735 ± 846 reads per individual (range: 509–5000 reads). We obtained 83 different MHC class-II alleles. All detected alleles were of the same length. The number of alleles per individual was 3.38 ± 0.83 (mean ± s.d.; range: 1–7).

*Chick MHC-diversity*

Several measures of MHC-diversity have been used across studies (Lenz et al., 2013; Radwan et al., 2012; Sepil, Lachish, & Sheldon, 2013). The most widely used proxy of MHC-diversity is the number of different MHC alleles possessed by an individual (e.g. Huchard, Knapp, et al., 2010; Kalbe et al., 2009; Roved et al., 2018). This measure relates to the “heterozygote advantage” (or overdominance) hypothesis. This hypothesis posits that heterozygous individuals should have a selective advantage over homozygous individuals, because they have a higher number of different MHC molecules, and thus can bind a higher number of antigens (Doherty & Zinkernagel, 1975). Wakeland et al. (1990) later proposed the "divergent allele advantage" hypothesis, which posits that, in heterozygous individuals, those with higher degree of functional divergence between alleles should have a selective advantage because their MHC molecules can bind a broader range of antigens. Several studies on a single MHC locus have thus used the functional divergence between two alleles to estimate MHC-diversity (Pierini & Lenz, 2018). Studies considering several MHC loci and using functional divergence to estimate MHC diversity are scarce. A few of them used the average functional divergence between all alleles and considered that individuals with higher mean divergence should be advantaged (Lenz, Wells, Pfeiffer, & Sommer, 2009; Schwensow, Eberle, & Sommer, 2010). However, the "divergent allele advantage" is expected to work in concert with the "heterozygote advantage" (Wakeland et al., 1990) and, when considering several loci, this estimate of MHC-diversity is, therefore, unlikely to be strongly correlated with the range of antigens bound by all alleles together. A few studies have thus used the degree of divergence over all loci to estimate MHC-diversity (Grieves, Gloor, Bernards, & MacDougall-Shackleton, 2019; Huchard et al., 2013; Leclaire et al., 2019; Radwan et al., 2012). This measure is expected to estimate the range of antigens recognized by MHC molecules, regardless of whether a high range is due to the possession of many alleles that are somewhat divergent or to the possession of a few alleles that

are very divergent. In our study, we decided, therefore, to estimate MHC-II diversity as the degree of divergence between alleles over all loci. In order to make comparison with other studies, we also carried out analyses on the number of functional MHC-II alleles and on the mean MHC-II divergence. These analyses are reported in the supporting information. Briefly, no effect of allele number or mean divergence on fitness traits were detected except for a sex-specific effect of allele number on tick infection and a weak effect of the square of divergence on growth (see supporting information for more details).

We used the Faith's diversity index to estimate MHC-II diversity as the degree of divergence of alleles across all loci (as in Grieves et al., 2019 and Leclaire et al., 2019). We first translated MHC-II DNA sequences into amino acid sequences, and considered DNA sequences as functionally identical if they had the same amino-acids in the peptide-binding regions (PBRs; inferred from Leclaire et al., 2014). Non-PBR sites were characterized by a low nucleotide diversity and codons with no significant excess of non-synonymous substitutions (Leclaire et al., 2014). We obtained a total of 68 functional alleles. The mean number of functional alleles per individual was 3.31 ± 0.78 (mean ± s.d.; range: 1-7; supporting information Figure S1) and did not significantly vary among years (Kruskal–Wallis, $U = 4.89$, $df = 8$, $p = 0.77$; supporting information Figure S2). Then, following the approach of Schwensow et al. (2007), we described the chemical binding properties of each amino acid in the PBRs with the Sandberg's five physico-chemical descriptors (z-descriptors; Sandberg, Eriksson, Jonsson, Sjostrom, & Wold, 1998). Following an approach adapted from Strandh et al. (2012), we used this Sandberg matrix to construct an alternative maximum-likelihood phylogenetic tree with “Rcontml” in the R package *Rphylip* (Revell & Chamberlain, 2014). This tree represents clusters of functionally-similar MHC-II sequences (see supporting information Figure S3) and was used as a reference to calculate the functional diversity of an

individual as the minimum total length of all the branches required to span its MHC-II alleles (i.e. Faith's phylogenetic diversity; Faith, 1992) with the R function "pd" in the *picante* R package (Kembel et al., 2010). In other words, for each additional allele, only the part of the peptide-binding characteristics that is not share with other alleles is summed. Faith's MHC-II diversity was significantly and positively correlated with both the number of functional MHC-II alleles (Pearson correlation, $t = 8.90$; $r = 0.32$; $p < 0.001$; $n = 699$) and the MHC-II divergence (Pearson correlation, $t = 2.89$; $r = 0.11$; $p = 0.004$; $n = 697$). Chick Faith's MHC-II diversity varied from 0.89 to 9.81 (mean ± s.d.: 6.01 ± 1.19; supporting information Figure S4) and did not significantly vary among years (Kruskal–Wallis, $U = 4.24$, $df = 8$, $p = 0.83$; supporting information Figure S5).

**Chick fitness parameters**

*Survival*

To record disappearance and death, all nests were checked twice daily (9:00 and 18:00) throughout the season until we left the study site (August 15$^{th}$).

*Morphological measurements and growth*

Chicks were measured every 5 days from hatching to the age of 35 days. We measured body mass to the nearest 0.1 g using an electronic scale, tarsus length to the nearest 0.1 mm with a caliper and wing length to the nearest 1 mm with a wing ruler.

We estimated body mass and size growth rates over 35 days by calculating the maximum slope of a logistic growth curve between morphological measures and age (T. Merkling et al., 2012) using the *grofit* package in R (Kahm, Hasenbrink, Lichtenberg-Frate, Ludwig, & Kschischo, 2010). Chick size was estimated by taking the scores of the first principal component analysis on wing and tarsus length at 0, 5, 10, 15, 20, 25, 30 and 35 days together.

Because such measurement necessarily excludes chicks that were not measured up to 35 days old, we also estimated growth rates over the first 10 days by calculating the slope of the linear regression between the morphological measures and age (T. Merkling et al., 2014; T. Merkling et al., 2016).

*Tick infection*

For each chick hatched in the 2008, 2009 and 2010 breeding seasons, we recorded the number of attached ticks (*Ixodes uriae*) every 5 days from 5 to 30 days through visual examination and palpation (E. Danchin, 1992). *I. uriae* is the only tick species known to infect kittiwakes on Middleton Island (BM Williams, personal communication). Ticks generally start to feed on 5-day-old chicks and parasitism may continue until fledging (Boulinier & Danchin, 1996).

**Statistical analysis**

*Sample size*

In all statistical analyses, eggs were excluded when their handling for other experimental purposes could have affected fitness. Because our study aimed at investigating the effect of chick (or embryo) sex on MHC-II-fitness relationships, we excluded unsexed chicks (or embryos) from the analyses (n = 94 individuals). These filters, together with the fact that tick infections were checked only over 3 years, led to different sample sizes for each fitness parameter: 17 non-hatched embryos and 429 chicks were used for survival analyses, 680 chicks for the analyses on condition and size at hatching, 292 chicks for growth rate analyses over the first 10 days, 209 chicks for growth rate analyses over 35 days and 138 chicks for tick infection analyses.

*Model selection*

We used an AICc-based information-theoretic approach to test how MHC-II diversity was associated with fitness-related traits (Burnham & Anderson, 2004; Burnham, Anderson, & Huyvaert, 2011). For each fitness-related trait, we built a set of candidate models corresponding to biologically plausible hypotheses explaining the response variable. Each set of candidate models also included a null model (intercept only) and when a model included an interaction, we always considered an additive model (i.e. without the interaction). We selected the best models based on their ΔAICc (i.e. the difference between the AICc of a given model and the AICc of the best model) by keeping every model with $\Delta AICc \leq 4$. This cut-off can be considered as conservative and retains the true best model with an approximate 95% confidence (Richards, 2005). Using these best models, we computed natural model-averaged parameter estimates, standard errors and 95% confidence intervals without shrinkage; i.e. parameter estimates of each variable were averaged using only the models with $\Delta AICc < 4$ in which they appear (Nakagawa & Freckleton, 2011). Model selection and averaging were conducted using the *MuMIn* package (Bartoń, 2018) and based on maximum likelihood estimation (see supporting information for an outline of all models). All statistical analyses were performed with R 3.5.2 (R Core Team, 2018).

For each fitness-related trait, we built a set of models that included MHC-II diversity, the square of MHC-II diversity, sex, hatching order and two- and three-way interactions between sex, hatching order and MHC-II diversity. We standardized continuous variables in all analyses, checked for collinearity issues and included clutch identity (ID), pair ID and year as random effects in the models. The pair ID random effect was however removed from models because associated variance estimates were virtually zero. We checked for normal distribution of random effects using the best model in model selection. We detected a significant association between sex and functional MHC-II diversity at hatching (t-test: $t = -2.75$; $p = 0.006$; $n = 680$

chicks) and at 35 days old (t-test: t = -2.10; p = 0.037; n = 209 chicks). At hatching and at 35 days-old, male chicks were less MHC-II diverse than female chicks (mean ± s.d.: males at hatching: 5.90 ± 1.48; males at 35 days old: 5.98 ± 1.20; females at hatching: 6.14 ± 1.31; and females at 35 days old: 6.30 ± 1.15). We thus ran separate models for male and female chicks for all analyses.

*Survival at the nest*

We tested for the effect of MHC-II diversity on chick survival at the nest using Cox proportional hazard mixed-effect models in the R package *coxme* (Therneau, 2018). We considered chick survival between 0 and 35 days (i.e. before fledging; Coulson & White, 1958; Maunder & Threlfall, 1972). Sixteen chicks younger than 35 days (mean ± s.d. : 30.6 ± 4.3 days old) were still alive when we left the study site. To be confident that survivors included in analyses fledged after our departure, we excluded those sixteen chicks because chicks' likelihood to fledge is very high once they reach 35 days old (Barrett & Runde, 1980; T. Merkling et al., 2014). *Body condition and size at hatching and growth*

We used linear mixed models (LMMs) in the *lme4* R package (Bates, Machler, Bolker, & Walker, 2015) to test for the effects of MHC-II diversity on body condition and size at hatching and on body mass and size growth rates. Chick size at hatching was included in the model built for body mass at hatching, which can thus be interpreted as size-adjusted body mass, or body condition (Garcia-Berthou, 2001). We checked for normality and homoscedasticity of residuals using the best model in model selection.

*Tick infection*

We investigated the association between MHC-II diversity and three variables linked to tick infection. First, we determined whether MHC-II diversity was associated with the probability

and the timing of first infection by ticks during the nestling stage (between 5 and 30 days old; n = 138 chicks) by fitting Cox proportional hazard mixed-effect models in the R package *coxme* (Therneau, 2018). Chicks that were not infected by ticks during this period were right-censored (n =28). Second, we focused on quantitative resistance by testing whether MHC-II diversity was associated with the maximum number of ticks chicks had during the nestling period (n = 110 chicks, excluding those without ticks) using zero-truncated models with a Poisson distribution using the *glmmTMB* R package (Brooks et al., 2017). If models with a zero-truncated Poisson distribution were overdispersed, we compared their fit to the fit of models with zero-truncated negative binomial 1 and zero-truncated negative binomial 2 distributions. We pooled the values over 15 to "15+" to reduce overdispersion (i.e. four chicks had more than 15 ticks). Finally, we determined whether MHC-II diversity was associated with the probability and the timing of tick loss during the nestling stage (n = 86 chicks) by fitting Cox proportional hazard mixed-effect models in the R package *coxme* (Therneau, 2018). Chicks without ticks (n = 28) or discontinuously infected by ticks (n = 24) were excluded from this analysis. Chicks that still had ticks at the end of the observation period were right-censored. We removed the clutch ID random effect from models because associated variance estimates were virtually zero.

## RESULTS

### Survival at the nest

Among 430 monitored eggs, 142 chicks (33%) died before reaching 35 days of age. In single-sex models, the interaction between MHC-II diversity and hatching order was significant only in females (estimate ± s.e. = -0.58 ± 0.28, 95% CI: -1.13, -0.04; supporting information Tables S1, S2). Mortality significantly decreased with increasing MHC-II diversity in female B-chicks,

but not in female A-chicks, while we detected no significant effect of MHC-II diversity on mortality in males (Figures 1, S6).

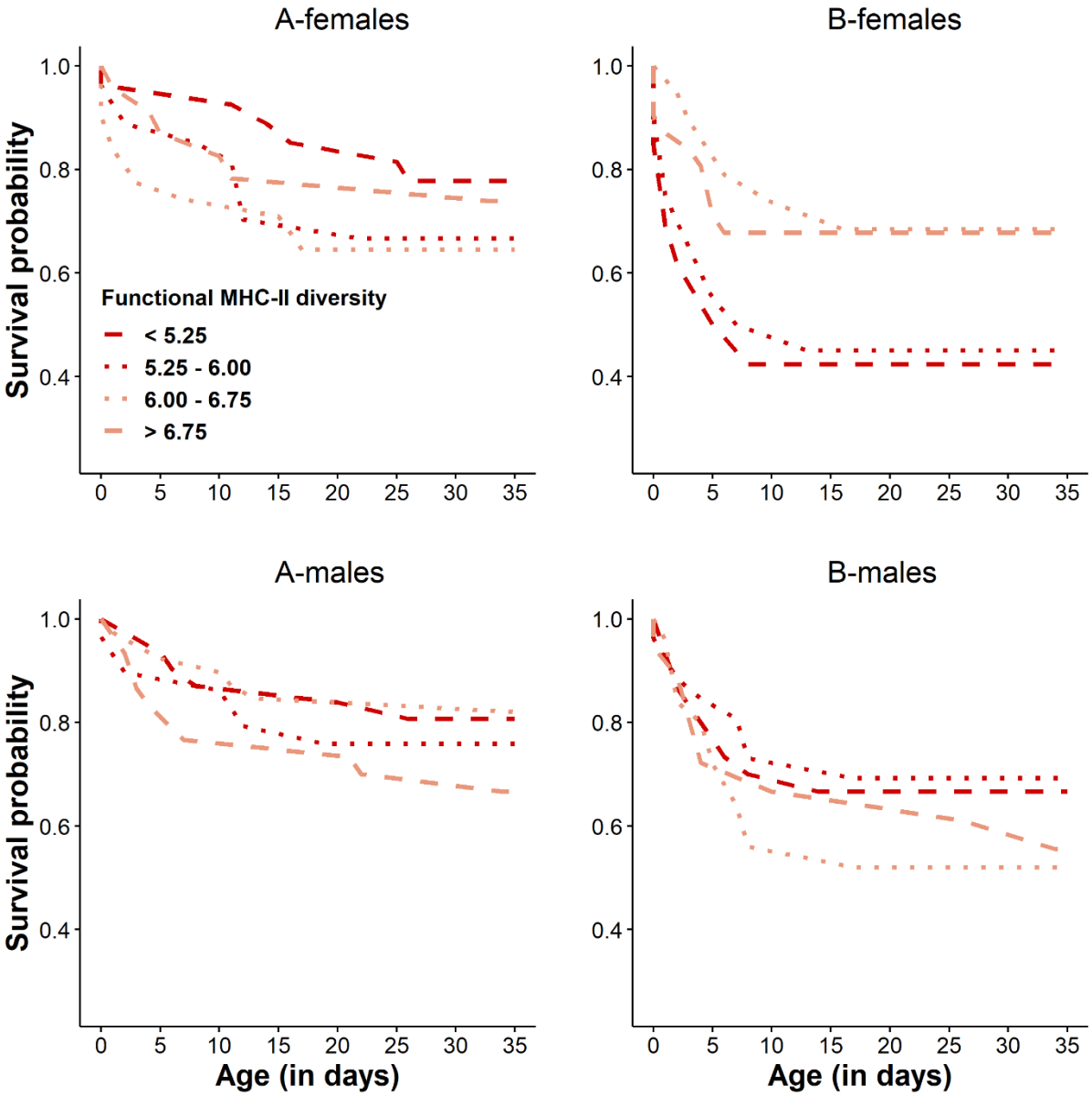


**Figure 1.** Chick survival probability during the nestling stage according to functional MHC-II diversity for female A- (n = 107), female B- (n = 96), male A- (n = 128) and male B-chicks (n = 99). Although functional MHC-II diversity was analyzed as a continuous variable (see text), it is displayed here as a categorical variable for illustrative purposes. We categorized MHC-II diversity in four groups using quartiles of the whole data set used for survival analyses (n = 430). The colors and line types represent MHC-II diversity, with red corresponding to the first (dashed line) and second (dotted line) quartiles (i.e. low MHC-II diversity) and pink to the third (dotted line) and fourth (dashed line) quartiles (i.e. high MHC-II diversity). See supporting information (Figure S10) for a different display (heatmap).

**Body condition and size at hatching**

Body condition at hatching was not significantly associated with functional MHC-II diversity (Tables S3, S4). Although there was a negative trend in males, size at hatching was not significantly associated with MHC-II diversity (females: estimate ± s.e. = 0.01 ± 0.06, 95% CI: -0.10, 0.12; males: estimate ± s.e. = -0.10 ± 0.05, 95% CI: -0.20, 0.01; Tables S5, S6).

**Growth**

In females only, body mass growth rate (females: estimate ± s.e. = 0.26 ± 0.09, 95% CI: 0.08, 0.43; males: estimate ± s.e. = -0.001 ± 0.08, 95% CI: -0.15, 0.15; Tables S7, S8, Figure 2A) and body size growth rate over the first 10 days (females: estimate ± s.e. = 0.24 ± 0.09, 95% CI: 0.06, 0.42; males: estimate ± s.e. = 0.06 ± 0.07, 95% CI: -0.08, 0.20; Tables S9, S10, Figure 2B) were significantly and positively associated with MHC-II diversity. There was also a significant effect of the interaction between the square of MHC-II diversity and hatching order on body size growth rate in females (estimate ± s.e. = 0.26 ± 0.11, 95% CI: 0.05, 0.47; Table S9). However, this interaction became non-significant after removing one A-female with high MHC-II diversity and moderate size growth rate (estimate ± s.e. = 0.20 ± 0.13, 95% CI: -0.06, 0.46; Table S11). Similar results were obtained for growth rates over 35 days (Tables S12-S15; Figure S7).

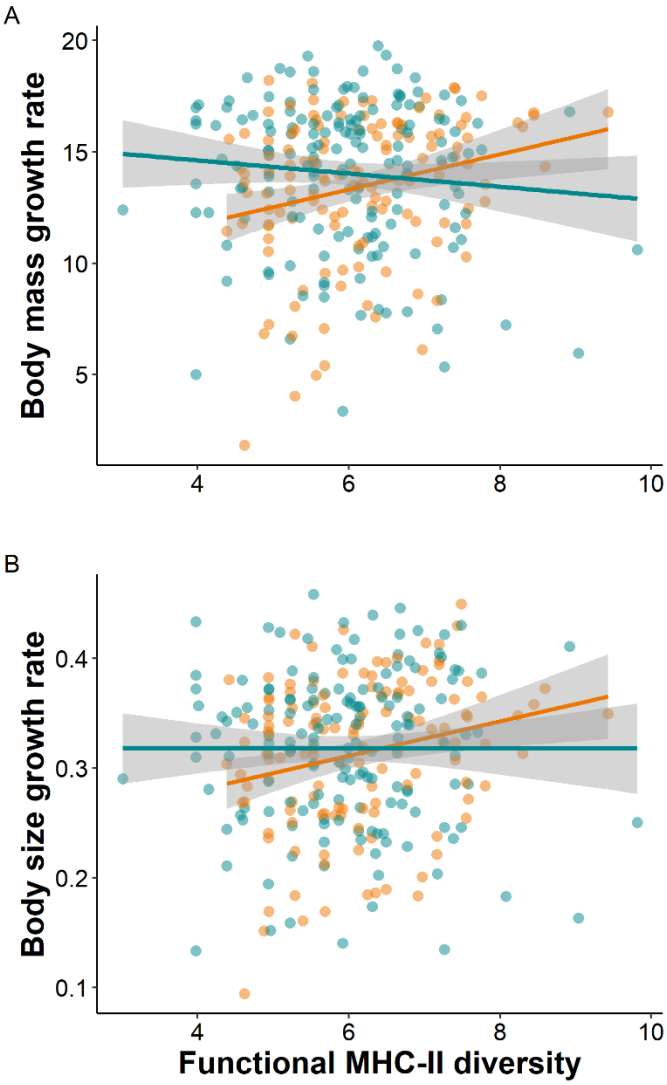


**Figure 2.** Growth rate of (A) chick body mass and (B) chick body size over the first 10 days according to functional MHC-II diversity in females (n = 131, in orange) and males (n = 160, in blue). Chick size was estimated by taking the scores of the first principal component analysis on wing and tarsus length. Growth rate was calculated as the slope of a linear regression between morphological measures and age (see methods for more details). One male with a very low MHC-II diversity (i.e. 0.88) has been removed to improve clarity of the figure (see Figure S10 for a figure including this male). Removing this male from analyses did not change the results. Regression lines were derived from single-sex models including functional MHC-II diversity of chicks as a fixed effect. Random effects (year and clutch ID) were not considered in the models used for graphic representations. Shaded areas represent confidence intervals.

**Tick infection**

In single-sex models, there was no significant association between MHC-II diversity and the age of first infection by ticks (females: estimate ± s.e. = -0.15 ± 0.14, 95% CI: -0.43, 0.12; males: estimate ± s.e. = -0.10 ± 0.14, 95% CI: -0.37, 0.17; Tables S16, S17) or the maximum number of ticks (females: estimate ± s.e. = -0.29 ± 0.22, 95% CI: -0.73, 0.15; males: estimate ± s.e. = -0.01 ± 0.26, 95% CI: -0.51, 0.51; Tables S18, S19). However, in females only, the probability to lose all ticks was positively and significantly associated with MHC-II diversity (females: estimate ± s.e. = 0.54 ± 0.20, 95% CI: 0.14, 0.94; males: estimate ± s.e. = -0.07 ±

0.21, 95% CI: -0.49, 0.34; Tables S20, S21). The more females were MHC-II diverse, the more likely and the faster they were to lose all ticks (Figures 3, 4). There was no significant effect of any other parameter on these three response variables (Tables S16-S21).

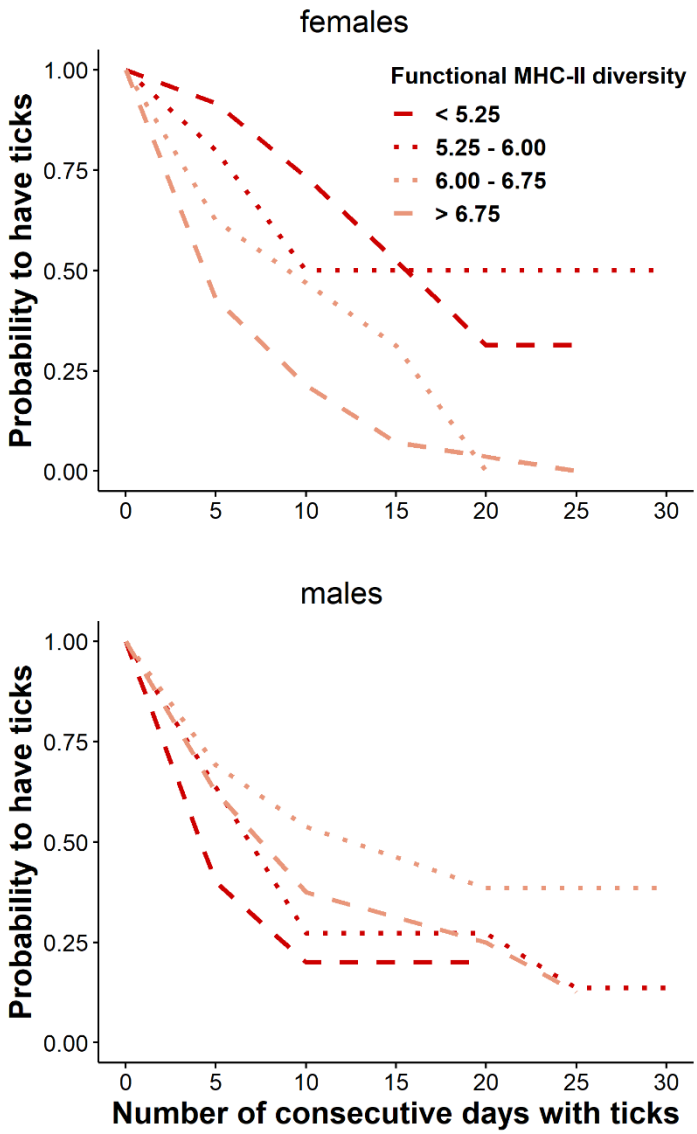


**Figure 3.** Probability of chicks to be infected according to infection duration and functional MHC-II diversity for females (n = 44) and males (n = 42). Although functional MHC-II diversity was analyzed as a continuous variable (see text), it is displayed here as a categorical variable for illustrative purposes. We categorized MHC-II diversity in four groups using quartiles of the whole data set used for tick analyses (n = 86). The colors and line types represent MHC-II diversity, with red corresponding to the first (dashed line) and second (dotted line) quartiles (i.e. low MHC-II diversity) and pink to the third (dotted line) and fourth (dashed line) quartiles (i.e. high MHC-II diversity).

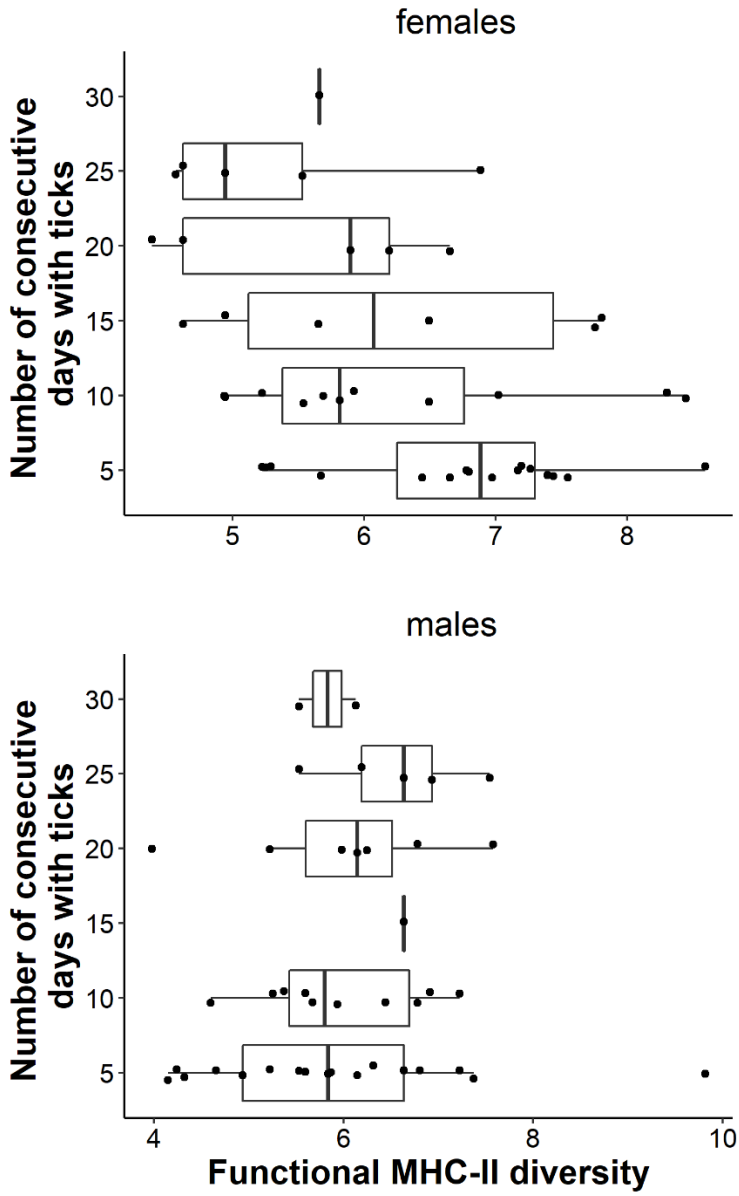


**Figure 4.** Boxplots of functional MHC-II diversity according to the number of consecutive days chicks were observed with ticks for females (n = 44) and males (n = 42).

## DISCUSSION

Although fitness advantages have been associated with different levels of MHC-II diversity in a wide range of species and populations (Bonneaud et al., 2004; Brouwer et al., 2010; Lenz et al., 2013; Thoss et al., 2011; Wegner, Reusch, et al., 2003), only a handful of studies have investigated the possibility that individuals within a population might benefit from different levels of MHC-II diversity. Yet, individuals can differ greatly in exposure and immune responses to parasites, leading to the expectation that they might not benefit from MHC-II

diversity the same way. In this study, we investigated whether, in kittiwake chicks, MHC-II-fitness associations depended upon sex and hatching order, two factors expected to modulate exposure and immune response to parasites. As expected, we detected positive associations between MHC-II diversity and female chick survival, but only in second-hatched female chicks. In contrast, no association between MHC-II diversity and survival was detected in male chicks. High MHC-II diversity was also associated with faster growth and tick loss in female chicks only. Our results suggest, therefore, that female chicks, especially those hatched in second position, benefit from maximal levels of MHC-II diversity while male chicks do not.

Several underlying mechanisms may explain the sex- and hatching rank-dependent effects of MHC-II diversity on fitness in kittiwake chicks. Because high MHC-II diversity provides resistance to a wider range of parasites and is associated with more efficient immune response, the fitness benefits associated with high MHC-II diversity in female chicks, particularly when hatched in second position, might be explained by weaker immune responses and/or higher exposure to parasites compared to males and A-chicks. Decreased immune response may result from higher levels of immunosuppressive sex hormones, such as androgens (S. L. Klein & Flanagan, 2016). Although higher levels of androgens have been found in the yolk of B-eggs compared to A-eggs in kittiwakes (Benowitz-Fredericks et al., 2013; J. Gasparini et al., 2007), sex-differences in circulating levels of androgens have not been investigated in kittiwake chicks. However, female chicks have been found to have higher levels of androgens than male chicks in several other species (Fargallo, Martinez-Padilla, Toledano-Diaz, Santiago-Moreno, & Davila, 2007 and references therein). Non-exclusively, weaker immune responses might also result from a trade-off between immunity and other competing functions that require metabolic resources (Zuk & Stoehr, 2002). In kittiwakes, female- and B-chicks are smaller (Merkling et al., 2012), in poorer condition (this study; see supporting

information Tables S3-S4, S22, S23; Figure S8) and suffer more from sibling aggressions than other chicks (Delaunay, 2018; White, Leclaire, et al., 2010) suggesting that they might be less competitive for food, and thus have a lower amount of resources to allocate to immune functions (Beldomenico & Begon, 2010; Beldomenico et al., 2008). Interestingly, when restricting our MHC-II-fitness analyses to broods where one egg did not hatch, thereby excluding the role of competition and aggression between siblings in driving MHC-II-fitness associations, we did not find any effect of MHC-II diversity on survival in female B-chicks (n = 49 females) and on growth rate (n = 34 females) and tick loss (n = 14 females) in female chicks (supporting information Tables S24-S27). Although these results must be taken with caution because of the reduced sample size, they suggest that sibling interactions are a potential driver of sex- and rank-specific effects of MHC-II diversity on fitness in this species.

A surprising finding of our study was that female chicks had, on average, a higher MHC-II diversity than male chicks at hatching (mean ± s.d.: 6.14 ± 1.31 vs. 5.90 ± 1.48; see Materials and Methods). Yet, females and males should exhibit the same level of MHC-diversity as they share the genetic architecture of the MHC (i.e. MHC genes are located on autosomes; Murphy & Weaver, 2017). Sex-difference in mean MHC-diversity has already been reported in humans, rats and mice, with increased MHC-diversity in newborn males compared to newborn females (Dorak et al., 2002, and references therein). Proposed mechanisms include selective fertilization, egg resorption and embryo loss (Dorak et al., 2002). Selective fertilization and embryo loss have been associated with both the degree of MHC-similarity between parents (Lenz, Hafer, Samonte, Yeates, & Milinski, 2018; C Ober, Hyslop, Elias, Weitkamp, & Hauck, 1998; Wedekind et al., 1996; Zhu, Wan, Zhang, & Fang, 2019) and with the sex of the embryo (or the sex chromosome of the gametes; Navara, 2018). However, whether they can be affected by these two parameters in interaction remains largely unknown. In our dataset, female

hatchlings were still more MHC-II diverse than males when we considered only clutches with no egg loss (mean ± s.d.: 6.20 ± 1.12 vs 5.93 ± 1.15; t-test: t = -2.55; p = 0.01; n = 448 chicks), suggesting that the sex-difference in hatchlings MHC-II diversity is not triggered by a sex-specific effect of MHC-II diversity on post-laying embryo mortality. Regardless of the underlying mechanism, MHC-II-similar parents, that are more likely to produce chicks with low MHC-II diversity (Setchell, Abbott, Gonzalez, & Knapp, 2013), might benefit from avoiding the fitness costs associated with the production of low MHC-II diverse daughters.

While our results are consistent with a direct effect of MHC-II on fitness-related traits, some methodological limitations and alternative explanations must be acknowledged. First, we amplified exon 2 of the MHC-II because it codes for the majority of amino acids that form the peptide-binding groove in model species like humans (J. H. Brown et al., 1993; Saper, Bjorkman, & Wiley, 1991) and because it has been the focus of most MHC research in non-model avian species (Minias, Pikus, Whittingham, & Dunn, 2018). However, both exon 2 and exon 3 encode the peptide binding grooves on MHC-II molecules. Second, we cannot rule out the possibility that variation at other genes may partly explain our results. For instance, MHC-II alleles might be in linkage disequilibrium with other MHC genes (e.g. MHC class I genes), owing to the compact architecture of the avian MHC (Hess & Edwards, 2002). Third, our results may possibly be explained by a broader effect of inbreeding on fitness, as variations at MHC genes can be correlated with genome-wide genetic variation depending on the life history, the dispersal ability and the breeding system of the study species (Sommer, 2005). Several studies reported sex-specific effects of inbreeding on fitness related traits (Billing et al., 2012; T. Coulson, Albon, Slate, & Pemberton, 1999; Rioux-Paquette et al., 2011). In hihis (*Notiomystis cincta*), the loss of inbred female embryos at a very early stage was proposed to explain increased heterozygosity in females later in the development (Brekke et al., 2010). When testing

for an association between MHC-II diversity and an estimate of genome-wide diversity (i.e. standardized heterozygosity; Coltman, Pilkington, Smith, & Pemberton, 1999) in a collection of 614 adults for which we had both MHC-II and microsatellite data (nine microsatellite loci; see this thesis, chapter 3 for details), we found however no significant correlation (Pearson correlation: $t = 0.27$; $r = 0.01$; $p = 0.79$; supporting information Figure S9). Studies including a higher number of MHC genes, other immune genes, and a better measure of overall genetic diversity are strongly encouraged to disentangle their effects on fitness in kittiwake chicks.

This study underscores the importance of considering traits that are expected to shape an individual's exposure and immune responses to parasites when predicting the association between MHC-diversity and fitness. Sex-specific effects of MHC-diversity on fitness have been mostly studied in polygynous species, with a positive association between MHC-diversity and survival or reproductive success found in adult males (Huchard, Knapp, et al., 2010; Roved et al., 2018; Sauermann et al., 2001; Schaschl et al., 2012). Here, we provide evidence for sex-specific associations between MHC-II diversity and fitness in the early life of a monogamous species, thus calling for further research in species or populations with differing life-history strategies. A recent study in adult Leach's storm-petrels (*Oceanodroma leucorhoa*), a monogamous seabird, found a positive association between MHC-diversity and reproductive success in adult females but not in males (Hoover et al., 2018). The underlying explanation was that males avoided low MHC-diverse females during mate choice but it was unclear whether reduced reproductive success of low MHC-diverse females also partly resulted from a direct, detrimental effect of reduced female quality on offspring viability. For instance, female petrels may be more likely to suffer from sexually transmitted infections than males, as shown in kittiwakes (Van Dongen et al., 2019; White, Mirleau, et al., 2010), thereby explaining sex-specific effects of MHC-diversity on fitness. Future investigations of sex-specific associations

between MHC-II diversity and post-fledging survival or reproductive success in kittiwakes represent an interesting avenue to explore whether the benefits of high MHC-II diversity found in female chicks persist in adult females. Importantly, ignoring early-life stages may yield only a partial picture of how MHC affects fitness if selection removes the less fit genotypes early in life, leaving only relatively high-quality individuals that survived long enough to be sampled. Such biased picture may in turn lead to a misunderstanding of the evolution of reproductive strategies.

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S.L.). M.P. was supported by a French doctoral scholarship. The authors declare no conflict of interest.

## SUPPLEMENTARY MATERIAL

### Supplementary figures

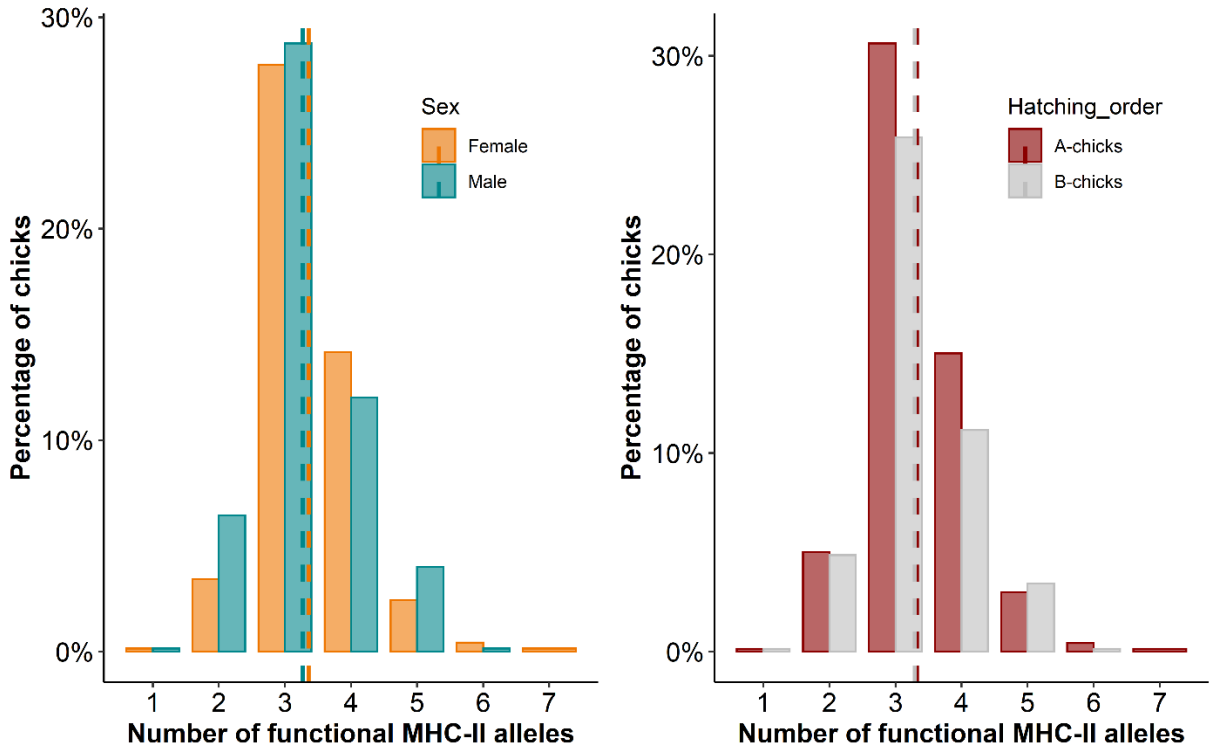


**Figure S1.** Distribution of the functional number of MHC-II alleles according to sex (left figure) with females in orange and males in blue, and hatching order (right figure) with A-chicks in pink and B-chicks in grey. Dashed lines represent mean number of functional MHC-II alleles per chick.

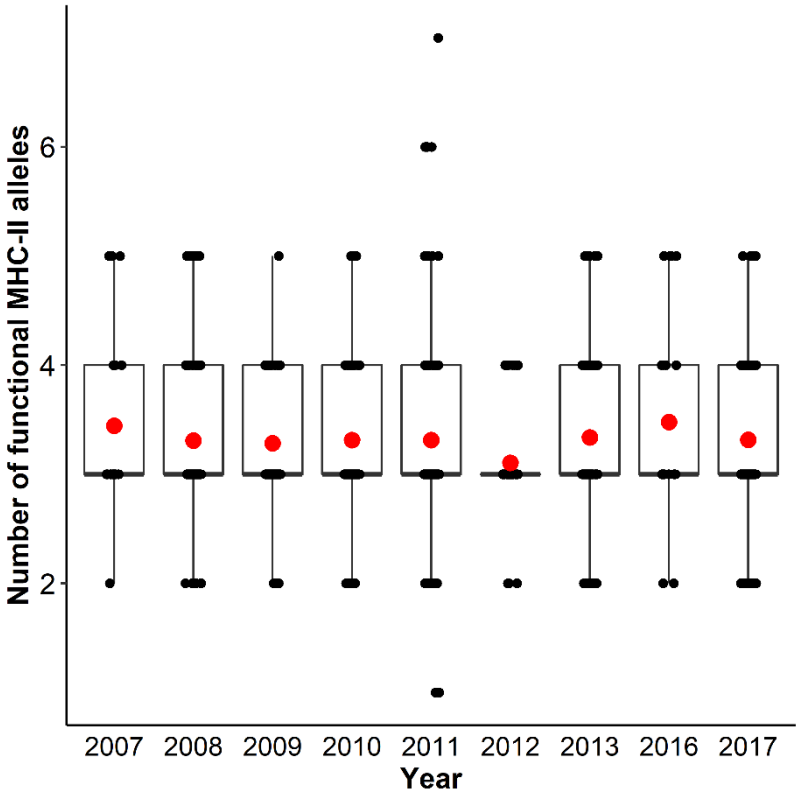


**Figure S2.** Boxplots of the number of functional MHC-II alleles in chicks according to year. Red dots represent mean number of functional MHC-II alleles per chick.

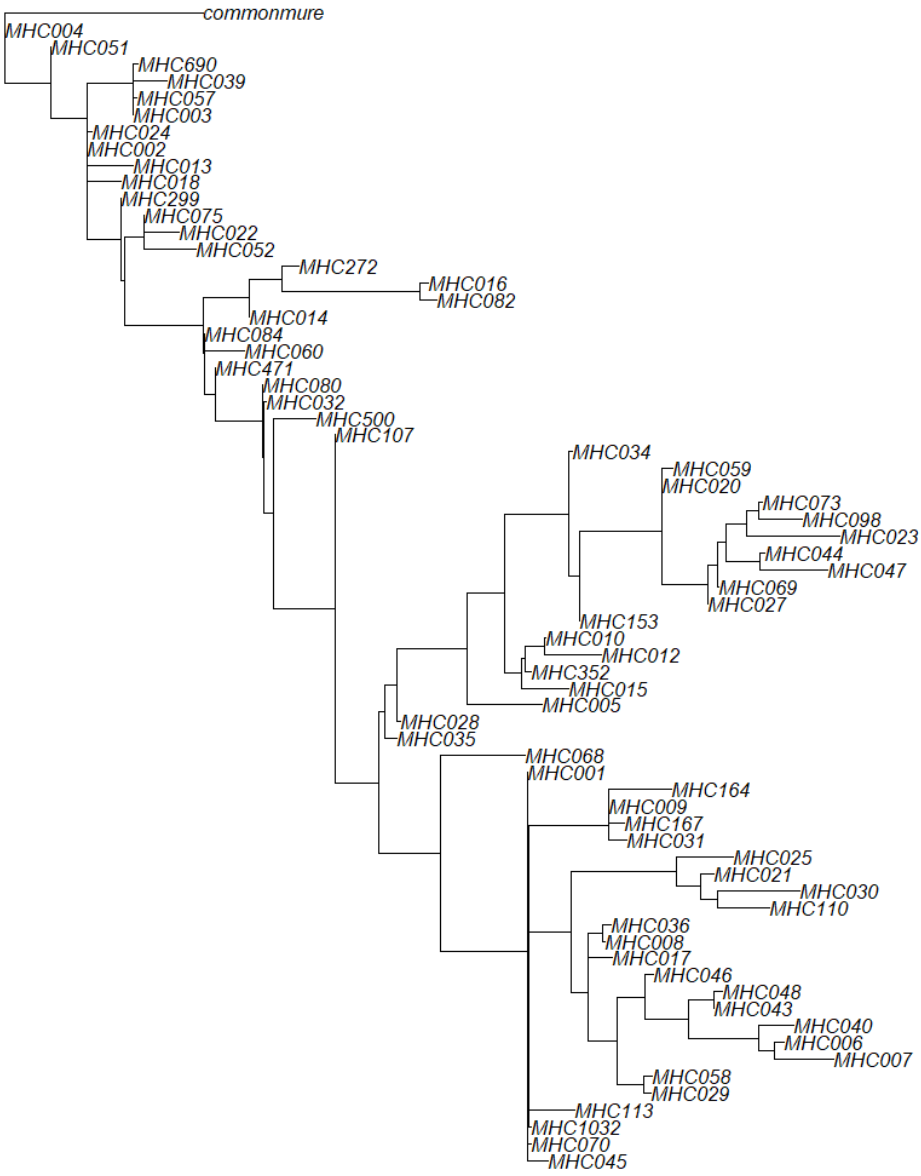


**Figure S3.** Maximum likelihood tree based on 68 functional MHC class IIB exon 2 sequences from the black-legged kittiwake (*Rissa tridactyla*) and one MHC class II sequence (Genbank EU326275.1) from the common murre (*Uria aalge*) as outgroup.

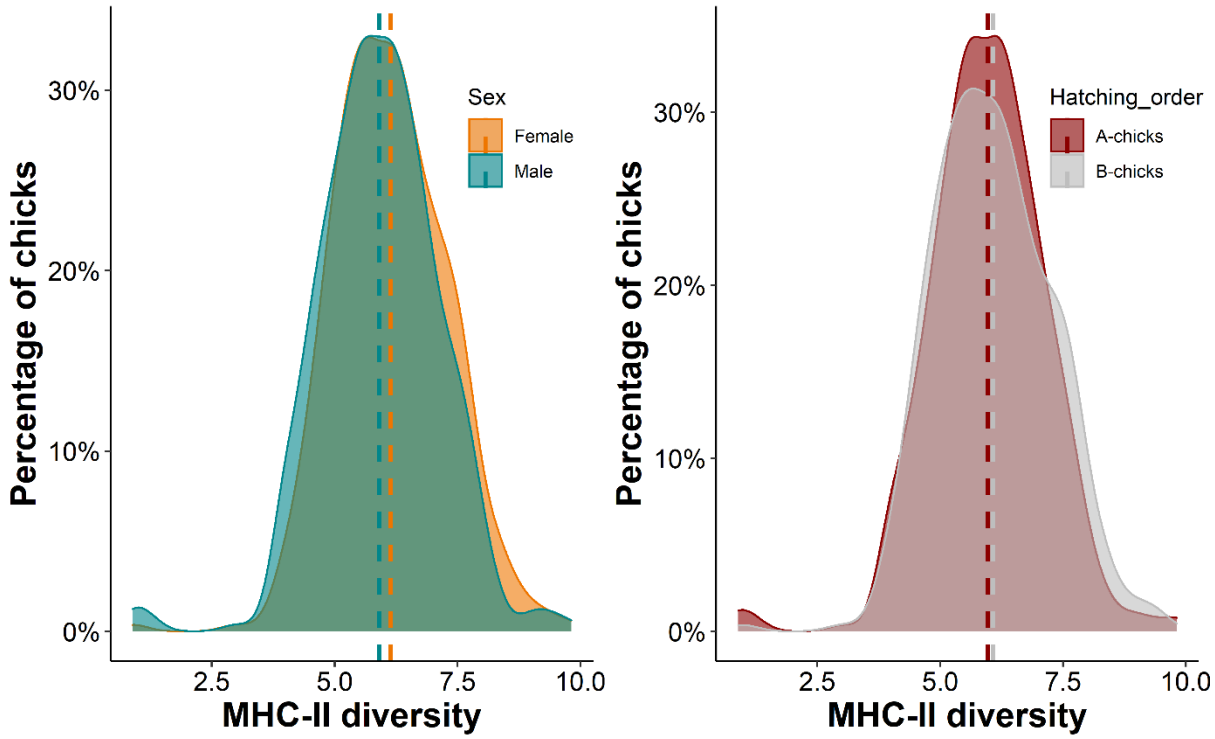


**Figure S4.** Distribution of Faith's MHC-II diversity in chicks according to sex (left figure) with females in orange and males in blue, and hatching order (right figure) with A-chicks in pink and B-chicks in grey. Dashed lines represent mean Faith's MHC-II diversity.

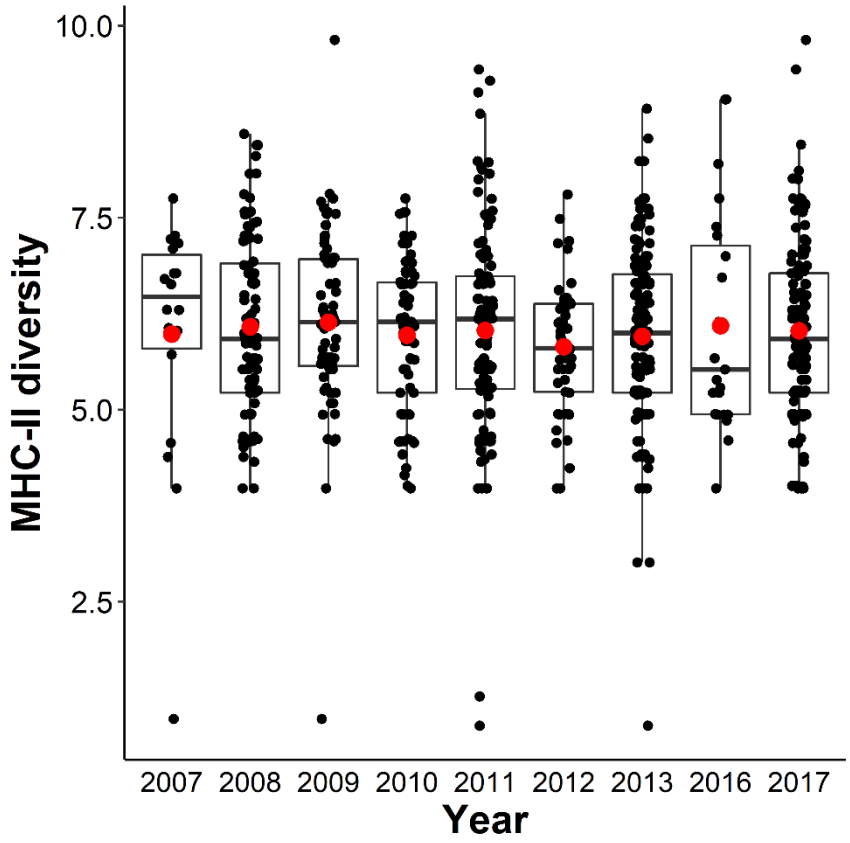


**Figure S5.** Boxplots of Faith's MHC-II diversity in chicks according to year. Red dots represent mean Faith's MHC-II diversity.

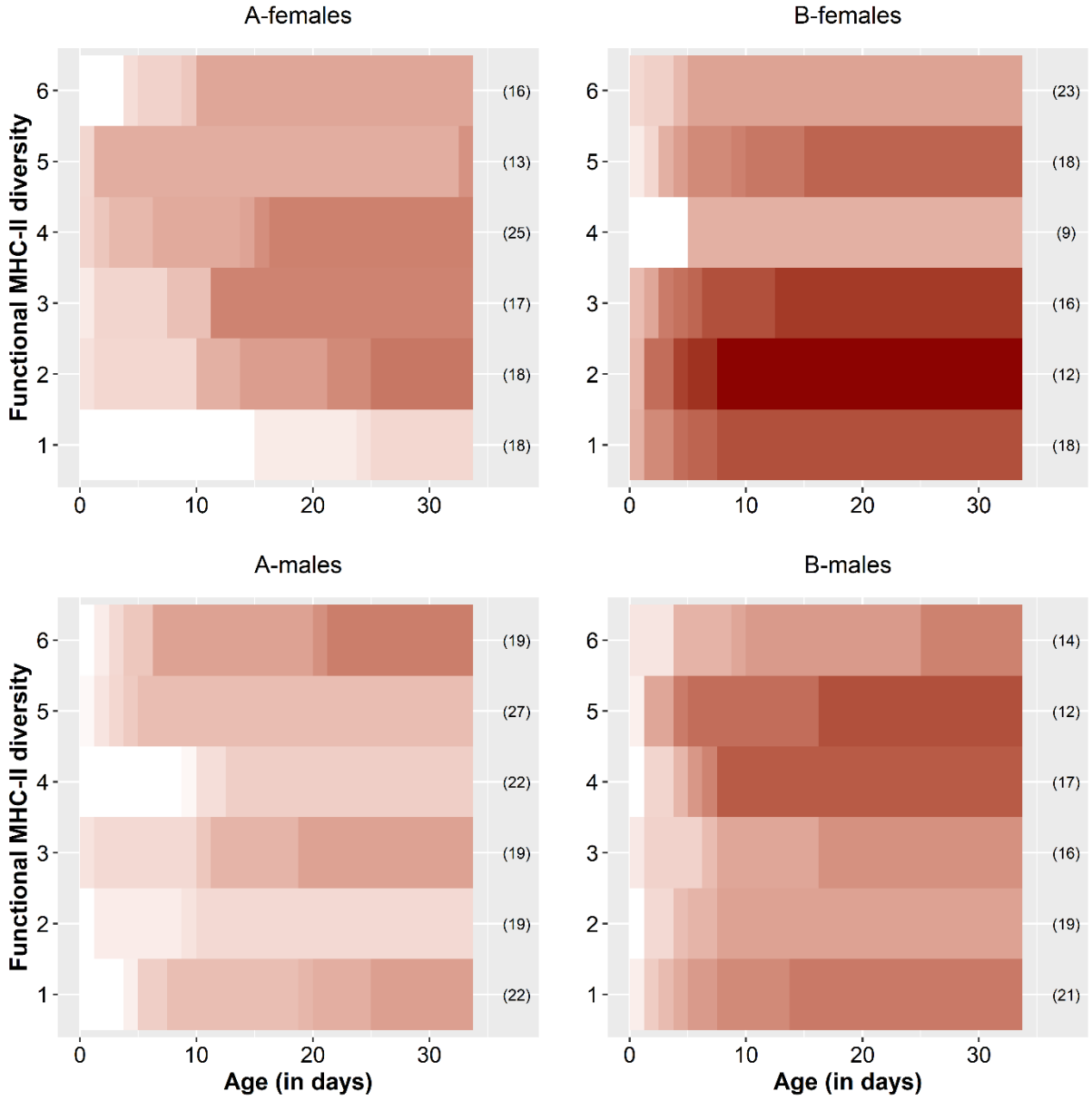


**Figure S6.** Chick mortality during the nestling stage according to age and Faith's MHC-II diversity for female A-, female B-, male A- and male B-chicks. Although Faith's MHC-II diversity was analyzed as a continuous variable (see text), it is displayed here as a categorical variable for illustrative purposes. We categorized Faith's MHC-II diversity in 6 groups using 6 quantiles from the whole data set used for survival analyses (n = 430 chicks). The color gradient represents chick mortality, with white corresponding to low mortality rate and red to high mortality rate. Numbers in parentheses correspond to the sample size for each category of Faith's MHC-II diversity.

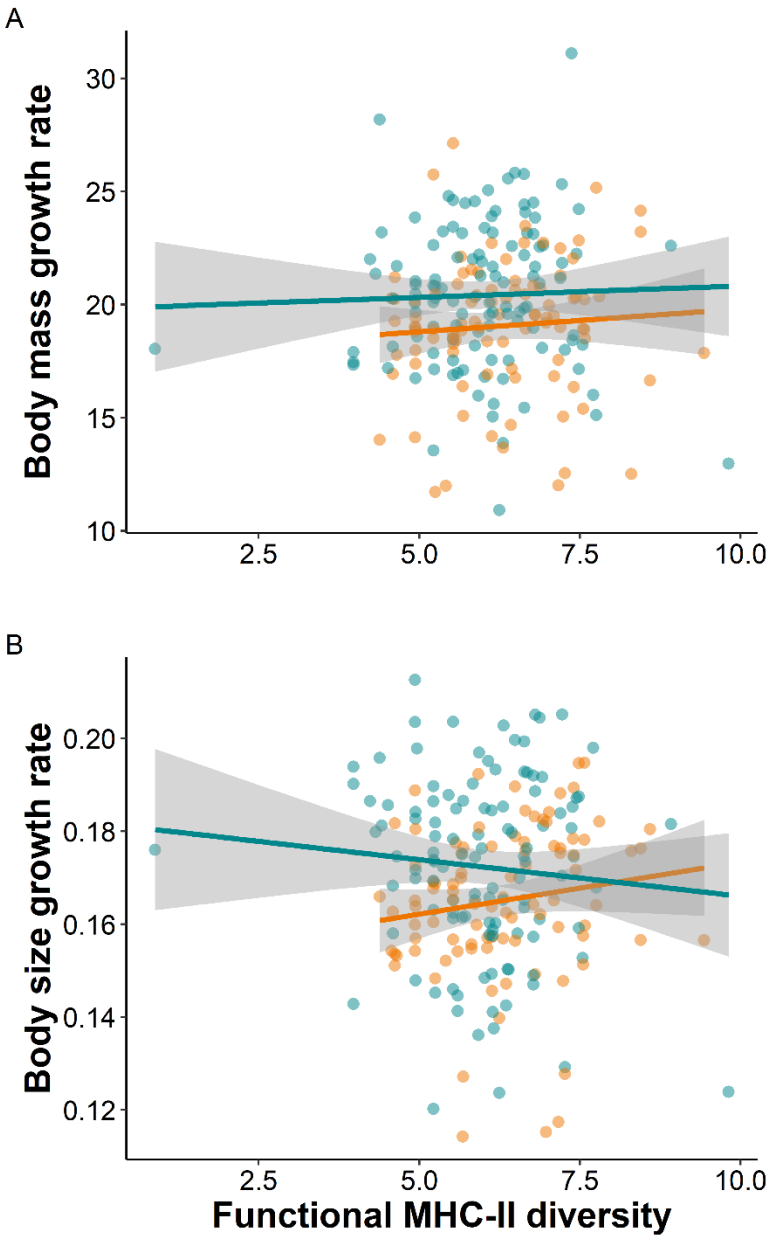


**Figure S7.** Growth rate of (A) chick body mass and (B) chick body size over 35 days according to Faith's MHC-II diversity for females (n = 94, in orange) and males (n = 115, in blue). Chick size was estimated by taking the scores of the first principal component analysis on wing and tarsus length. Size growth rate was calculated as the maximum slope of a logistic growth curve between morphological measures and age (see the main manuscript for more details). Regression lines were derived from single-sex models including Faith's MHC-II diversity of chicks as a fixed effect. Random effects (year and clutch ID) were not considered in the models used for graphic representations.

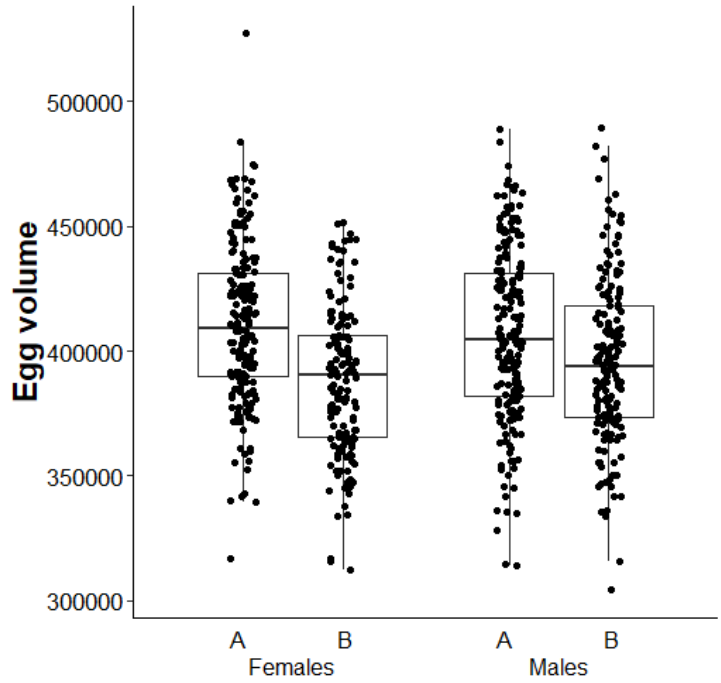


**Figure S8.** Boxplots of egg volume according to hatching order and sex. Egg volume was calculated as the volume of an ellipsoid (= π x (4/3) x egg width² x egg length). Egg width and egg length were measured to the nearest 0.01 mm at the longest and widest points.

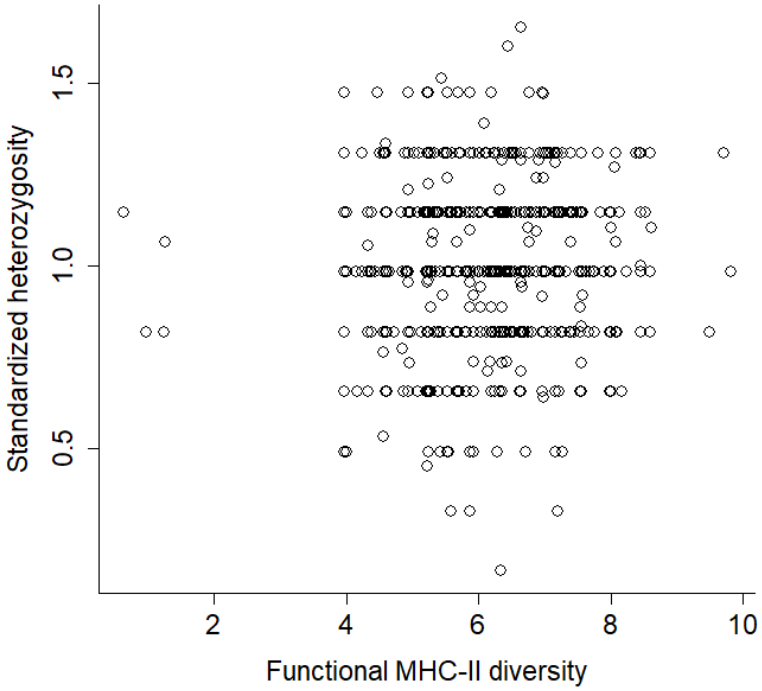


**Figure S9.** Correlation between Faith's MHC-II diversity and the standardized heterozygosity of adult kittiwakes (n = 614).

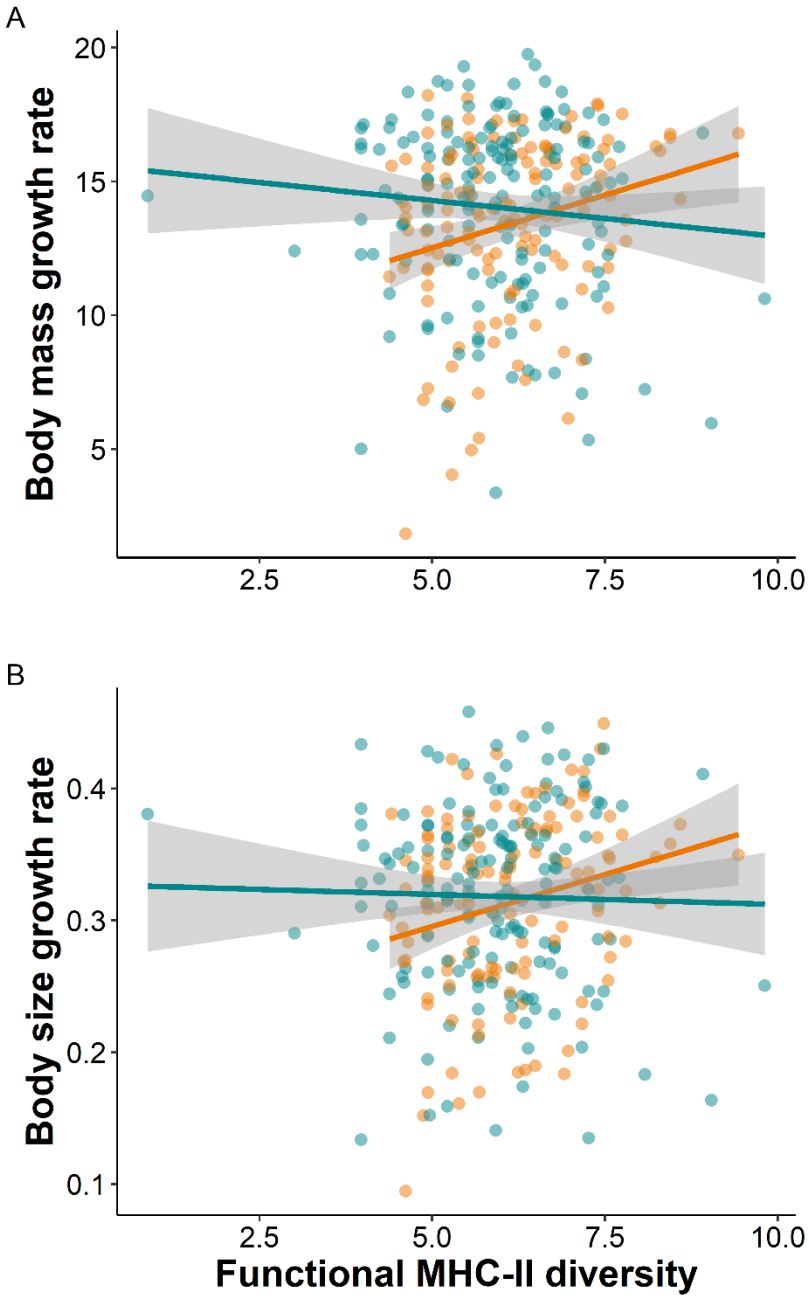


**Figure S10.** Growth rate of (A) chick body mass and (B) chick body size over the first 10 days according to Faith's MHC-II diversity for females (n = 131, in orange) and males (n = 161, in blue). This figure is the same than the Figure 3 in the main manuscript except it includes a male with a very low MHC-II diversity that was removed to improve clarity of the figure.

## Supplementary tables

**Table S1:** a) the subset of 4 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain female mortality, b) model-averaged estimates for all fixed parameters in this subset of models and c) variance and standard deviation (SD) associated with random effects in the best model. 'df' denotes the degrees of freedom; 'logLik' is the log-likelihood; 'AICc' is the AIC corrected for finite sample size; 'ΔAICc' is the difference between AICc of a given model to that of the best model; 'ωAICc' is the probability of each model given the data and the model set; 'Std. estimate' denotes the estimate of the standardized variable (effect size); 'SE' the conditional standard error of the parameter; 'Lower CI' and 'Upper CI' are the lower and upper bound of the 95% confidence interval. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| MHC-diversity * Hatching order | 9 | -343.29 | 707.19 | 0.00 | 0.52 |
| MHC-diversity * Hatching order + MHC-diversity^2 * Hatching order | 11 | -342.12 | 709.33 | 2.13 | 0.18 |
| Hatching order | 7 | -346.69 | 709.56 | 2.37 | 0.16 |
| MHC-diversity + Hatching order | 8 | -345.78 | 709.88 | 2.69 | 0.14 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| MHC-diversity | 0.122 | 0.246 | -0.360 | 0.604 |
| **B-chick[a]** | **0.797** | **0.290** | **0.231** | **1.369** |
| **MHC-diversity : B-chick[a]** | **-0.583** | **0.277** | **-0.127** | **-0.040** |
| MHC-diversity^2 | -0.349 | 0.304 | -0.945 | 0.246 |
| MHC-diversity^2 : B-chick[a] | 0.431 | 0.317 | -0.190 | 1.053 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.001 | 0.020 |
| Year | 1.874 | 1.369 |

**Table S2:** a) the subset of 4 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain male mortality, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table S1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order | 7 | -319.11 | 654.47 | 0.00 | 0.52 |
| MHC-diversity + Hatching order | 8 | -319.03 | 656.44 | 1.97 | 0.19 |
| MHC-diversity^2 + Hatching order | 8 | -319.09 | 656.57 | 2.10 | 0.18 |
| MHC-diversity^2 * Hatching order | 9 | -318.51 | 657.65 | 3.18 | 0.11 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| **B-chick[a]** | **0.930** | **0.260** | **0.420** | **1.439** |
| MHC-diversity | 0.057 | 0.119 | -0.176 | 0.290 |
| MHC-diversity^2 | -0.007 | 0.063 | -0.131 | 0.118 |
| MHC-diversity^2 : B-chick[a] | 0.087 | 0.092 | -0.093 | 0.267 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.001 | 0.020 |
| Year | 1.370 | 1.170 |

**Table S3:** a) the subset of 4 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain female chick body condition at hatching, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Chick size was included in all models. Abbreviations are described in Table S1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| MHC-diversity + Hatching order | 7 | -399.05 | 812.46 | 0.00 | 0.34 |
| Hatching order | 6 | -400.22 | 812.70 | 0.24 | 0.30 |
| MHC-diversity * Hatching order | 8 | -398.38 | 813.23 | 0.77 | 0.23 |
| MHC-diversity^2 + Hatching order | 7 | -400.04 | 814.44 | 1.99 | 0.13 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | 0.173 | 0.134 | -0.090 | 0.437 |
| MHC-diversity | -0.090 | 0.057 | -0.202 | 0.022 |
| **B-chick[a]** | **-0.558** | **0.085** | **-0.724** | **-0.391** |
| **Size** | **0.365** | **0.048** | **0.270** | **0.459** |
| MHC-diversity : B-chick[a] | 0.100 | 0.087 | -0.070 | 0.270 |
| MHC-diversity^2 | 0.016 | 0.027 | -0.038 | 0.069 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.293 | 0.542 |
| Year | 0.115 | 0.389 |

**Table S4:** a) the subset of 5 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain male chick body condition at hatching, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Chick size was included in all models. Abbreviations are described in Table S1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| MHC-diversity^2 + Hatching order | 7 | -452.60 | 919.53 | 0.00 | 0.42 |
| Hatching order | 6 | -454.22 | 920.68 | 1.15 | 0.23 |
| MHC-diversity^2 * Hatching order | 8 | -452.55 | 921.51 | 1.99 | 0.15 |
| MHC-diversity + Hatching order | 7 | -453.77 | 921.86 | 2.33 | 0.13 |
| MHC-diversity * Hatching order | 8 | -453.36 | 923.13 | 3.60 | 0.07 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | 0.010 | 0.140 | -0.266 | 0.284 |
| MHC-diversity^2 | 0.041 | 0.024 | -0.005 | 0.088 |
| **B-chick[a]** | **-0.305** | **0.084** | **-0.471** | **-0.140** |
| **Size** | **0.377** | **0.047** | **0.285** | **0.469** |
| MHC-diversity^2 : B-chick[a] | -0.014 | 0.047 | -0.107 | 0.077 |
| MHC-diversity | -0.055 | 0.053 | -0.160 | 0.049 |
| MHC-diversity : B-chick[a] | 0.075 | 0.089 | -0.094 | 0.254 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.282 | 0.531 |
| Year | 0.132 | 0.363 |

**Table S5:** a) the subset of 6 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain female chick body size at hatching, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Null model | 4 | -447.02 | 902.17 | 0.00 | 0.30 |
| Hatching order | 5 | -446.09 | 902.37 | 0.20 | 0.27 |
| MHC-diversity^2 | 5 | -446.96 | 904.12 | 1.95 | 0.11 |
| MHC-diversity | 5 | -447.01 | 904.21 | 2.04 | 0.11 |
| MHC-diversity^2 + Hatching order | 6 | -446.03 | 904.33 | 2.16 | 0.10 |
| MHC-diversity + Hatching order | 6 | -446.06 | 904.39 | 2.22 | 0.10 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | 0.101 | 0.141 | -0.177 | 0.379 |
| B-chick[a] | -0.134 | 0.098 | -0.327 | 0.057 |
| MHC-diversity^2 | 0.011 | 0.031 | -0.051 | 0.073 |
| MHC-diversity | 0.011 | 0.054 | -0.096 | 0.117 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.370 | 0.609 |
| Year | 0.121 | 0.347 |

**Table S6:** a) the subset of 6 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain male chick body size at hatching, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| MHC-diversity | 5 | -494.15 | 998.47 | 0.00 | 0.43 |
| Null model | 4 | -496.04 | 1000.20 | 1.73 | 0.18 |
| MHC-diversity + Hatching order | 6 | -494.00 | 1000.23 | 1.77 | 0.18 |
| Hatching order | 5 | -495.84 | 1001.85 | 3.38 | 0.08 |
| MHC-diversity^2 | 5 | -495.98 | 1002.12 | 3.66 | 0.07 |
| MHC-diversity * Hatching order | 7 | -493.96 | 1002.24 | 3.77 | 0.07 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | 0.070 | 0.125 | -0.176 | 0.315 |
| MHC-diversity | -0.100 | 0.053 | -0.203 | 0.004 |
| B-chick[a] | -0.056 | 0.097 | -0.246 | 0.135 |
| MHC-diversity^2 | 0.009 | 0.025 | -0.041 | 0.059 |
| MHC-diversity : B-chick[a] | 0.028 | 0.102 | -0.172 | 0.228 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.207 | 0.455 |
| Year | 0.102 | 0.320 |

**Table S7:** a) the subset of 4 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain female chick body mass growth rate over the first 10 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table S1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| MHC-diversity | 5 | -165.84 | 342.15 | 0.00 | 0.36 |
| MHC-diversity + Hatching order | 6 | -164.82 | 342.31 | 0.16 | 0.34 |
| MHC-diversity * Hatching order | 7 | -164.45 | 343.81 | 1.66 | 0.16 |
| MHC-diversity * Hatching order + MHC-diversity^2 * Hatching order | 9 | -162.26 | 344.01 | 1.85 | 0.14 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.055 | 0.224 | -0.498 | 0.388 |
| **MHC-diversity** | **0.258** | **0.893** | **0.082** | **0.435** |
| B-chick[a] | -0.276 | 0.192 | -0.655 | 0.102 |
| MHC-diversity : B-chick[a] | -0.178 | 0.158 | -0.491 | 0.135 |
| MHC-diversity^2 | -0.090 | 0.106 | -0.300 | 0.120 |
| MHC-diversity^2 : B-chick[a] | 0.232 | 0.129 | -0.023 | 0.486 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.000 | 0.000 |
| Year | 0.333 | 0.577 |

**Table S8:** a) the 9 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain male chick body mass growth rate over the first 10 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table S1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order | 5 | -196.77 | 403.94 | 0.00 | 0.24 |
| Null model | 4 | -197.94 | 404.13 | 0.20 | 0.22 |
| MHC-diversity^2 + Hatching order | 6 | -196.45 | 405.44 | 1.50 | 0.11 |
| MHC-diversity^2 | 5 | -197.58 | 405.55 | 1.61 | 0.11 |
| MHC-diversity * Hatching order | 7 | -195.48 | 405.69 | 1.76 | 0.10 |
| MHC-diversity + Hatching order | 6 | -196.65 | 405.85 | 1.91 | 0.09 |
| MHC-diversity | 5 | -197.85 | 406.08 | 2.14 | 0.08 |
| MHC-diversity^2 * Hatching order | 7 | -196.23 | 407.20 | 3.26 | 0.05 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.295 | 0.262 | -0.812 | 0.221 |
| B-chick[a] | -0.186 | 0.123 | -0.428 | 0.056 |
| MHC-diversity^2 | -0.025 | 0.032 | -0.089 | 0.039 |
| MHC-diversity | -0.003 | 0.075 | -0.151 | 0.146 |
| MHC-diversity : B-chick[a] | -0.196 | 0.127 | -0.447 | 0.055 |
| MHC-diversity^2 : B-chick[a] | -0.060 | 0.090 | -0.237 | 0.117 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.218 | 0.467 |
| Year | 0.631 | 0.794 |

**Table S9:** a) the subset of 4 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain female chick body size growth rate over the first 10 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table S1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| MHC-diversity + Hatching order | 6 | -168.48 | 349.68 | 0.00 | 0.37 |
| MHC-diversity | 5 | -169.84 | 350.17 | 0.52 | 0.28 |
| MHC-diversity * Hatching order + MHC-diversity^2 * Hatching order | 9 | -165.56 | 350.60 | 0.96 | 0.23 |
| MHC-diversity * Hatching order | 7 | -168.44 | 351.79 | 2.15 | 0.12 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.043 | 0.203 | -0.444 | 0.358 |
| **MHC-diversity** | **0.239** | **0.090** | **0.061** | **0.416** |
| B-chick[a] | -0.292 | 0.171 | -0.623 | 0.050 |
| MHC-diversity^2 | -0.146 | 0.093 | -0.330 | 0.038 |
| MHC-diversity : B-chick[a] | -0.139 | 0.156 | -0.447 | 0.169 |
| **MHC-diversity^2 : B-chick[a]** | **0.264** | **0.106** | **0.054** | **0.473** |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.559 | 0.748 |
| Year | 0.212 | 0.461 |

**Table S10:** a) the subset of 8 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain male chick body size growth rate over the first 10 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order | 5 | -203.13 | 416.64 | 0.00 | 0.25 |
| Null model | 4 | -204.20 | 416.65 | 0.01 | 0.25 |
| MHC-diversity | 5 | -203.93 | 418.24 | 1.60 | 0.11 |
| MHC-diversity + Hatching order | 6 | -202.87 | 418.29 | 1.65 | 0.11 |
| MHC-diversity^2 | 5 | -204.18 | 418.76 | 2.12 | 0.09 |
| MHC-diversity^2 + Hatching order | 6 | -203.11 | 418.77 | 2.13 | 0.09 |
| MHC-diversity * Hatching order | 7 | -202.31 | 419.35 | 2.71 | 0.06 |
| MHC-diversity^2 * Hatching order | 7 | -202.93 | 420.60 | 3.96 | 0.03 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.302 | 0.249 | -0.794 | 0.189 |
| B-chick[a] | -0.191 | 0.133 | -0.453 | 0.072 |
| MHC-diversity | 0.058 | 0.071 | -0.082 | 0.199 |
| MHC-diversity^2 | 0.007 | 0.034 | -0.060 | 0.073 |
| MHC-diversity : B-chick[a] | -0.149 | 0.135 | -0.415 | 0.117 |
| MHC-diversity^2 : B-chick[a] | -0.056 | 0.094 | -0.242 | 0.130 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.113 | 0.336 |
| Year | 0.483 | 0.695 |

**Table S11:** a) the subset of 4 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain female chick body size growth rate over the first 10 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. One A-female with high MHC-diversity and moderate size growth rate was removed from this analysis. Abbreviations are described in Table S1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| MHC-diversity + Hatching order | 6 | -166.56 | 345.81 | 0.00 | 0.44 |
| MHC-diversity * Hatching order | 7 | -166.01 | 346.94 | 1.13 | 0.25 |
| MHC-diversity | 5 | -168.41 | 347.31 | 1.49 | 0.21 |
| MHC-diversity * Hatching order + MHC-diversity^2 * Hatching order | 9 | -164.59 | 348.67 | 2.86 | 0.10 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.043 | 0.198 | -0.435 | 0.349 |
| **MHC-diversity** | **0.257** | **0.096** | **0.068** | **0.446** |
| B-chick[a] | -0.273 | 0.142 | -0.555 | 0.008 |
| MHC-diversity : B-chick[a] | -0.171 | 0.144 | -0.455 | 0.114 |
| MHC-diversity^2 | -0.096 | 0.115 | -0.324 | 0.131 |
| MHC-diversity^2 : B-chick[a] | 0.201 | 0.133 | -0.062 | 0.464 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.570 | 0.755 |
| Year | 0.215 | 0.463 |

**Table S12:** a) the 7 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain female chick body mass growth rate over 35 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order | 5 | -127.24 | 265.16 | 0.00 | 0.26 |
| Null model | 4 | -128.56 | 265.56 | 0.40 | 0.21 |
| MHC-diversity * Hatching order | 7 | -125.39 | 266.08 | 0.93 | 0.16 |
| MHC-diversity + Hatching order | 6 | -126.84 | 266.5 | 1.49 | 0.12 |
| MHC-diversity^2 + Hatching order | 6 | -127.24 | 267.44 | 2.28 | 0.08 |
| MHC-diversity | 5 | -128.39 | 267.47 | 2.31 | 0.08 |
| MHC-diversity^2 | 5 | -128.47 | 267.63 | 2.47 | 0.08 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.184 | 0.255 | -0.690 | 0.322 |
| B-chick[a] | -0.331 | 0.192 | -0.711 | 0.050 |
| MHC-diversity | -0.153 | 0.144 | -0.131 | 0.438 |
| MHC-diversity : B-chick[a] | 0.319 | 0.183 | -0.683 | 0.046 |
| MHC-diversity^2 | 0.019 | 0.080 | -0.179 | 0.140 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.284 | 0.533 |
| Year | 0.377 | 0.614 |

**Table S13:** a) the subset of 6 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain male chick body mass growth rate over 35 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Null model | 4 | -159.13 | 326.62 | 0.00 | 0.33 |
| MHC-diversity^2 | 5 | -158.27 | 327.09 | 0.46 | 0.26 |
| Hatching order | 5 | -158.97 | 328.50 | 1.87 | 0.13 |
| MHC-diversity | 5 | -159.00 | 328.55 | 1.93 | 0.13 |
| MHC-diversity^2 + Hatching order | 6 | -158.12 | 329.02 | 2.39 | 0.10 |
| MHC-diversity + Hatching order | 6 | -158.86 | 330.50 | 3.88 | 0.05 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.112 | 0.186 | -0.481 | 0.257 |
| MHC-diversity^2 | -0.050 | 0.037 | -0.123 | 0.025 |
| B-chick[a] | -0.107 | 0.183 | -0.470 | 0.256 |
| MHC-diversity | 0.044 | 0.087 | -0.130 | 0.217 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.076 | 0.275 |
| Year | 0.183 | 0.428 |

**Table S14:** a) the subset of 3 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain female chick body size growth rate over 35 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table S1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| MHC-diversity + Hatching order | 6 | -116.41 | 245.81 | 0.00 | 0.53 |
| MHC-diversity * Hatching order | 7 | -115.78 | 246.88 | 1.07 | 0.31 |
| Hatching order | 5 | -118.79 | 248.27 | 2.49 | 0.16 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.010 | 0.263 | -0.532 | 0.513 |
| **MHC-diversity** | **0.229** | **0.110** | **0.012** | **0.447** |
| **B-chick[a]** | **-0.460** | **0.168** | **-0.794** | **-0.125** |
| MHC-diversity : B-chick[a] | -0.198 | 0.154 | -0.504 | 0.107 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.328 | 0.573 |
| Year | 0.411 | 0.641 |

**Table S15:** a) the subset of 7 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain male chick body size growth rate over 35 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order | 5 | -142.84 | 296.24 | 0.00 | 0.27 |
| Null model | 4 | -144.03 | 296.44 | 0.19 | 0.24 |
| MHC-diversity + Hatching order | 6 | -142.48 | 297.75 | 1.50 | 0.13 |
| MHC-diversity | 5 | -143.73 | 298.02 | 1.78 | 0.11 |
| MHC-diversity^2 + Hatching order | 6 | -142.82 | 298.42 | 2.19 | 0.09 |
| MHC-diversity^2 | 5 | -144.01 | 298.58 | 2.33 | 0.08 |
| MHC-diversity * Hatching order | 7 | -141.78 | 298.61 | 2.37 | 0.08 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.167 | 0.263 | -0.688 | 0.354 |
| B-chick[a] | -0.227 | 0.145 | -0.515 | 0.060 |
| MHC-diversity | -0.077 | 0.083 | -0.243 | 0.088 |
| MHC-diversity^2 | -0.007 | 0.033 | -0.073 | 0.059 |
| MHC-diversity : B-chick[a] | 0.019 | 0.161 | -0.127 | 0.511 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.352 | 0.593 |
| Year | 0.516 | 0.718 |

**Table S16:** a) the subset of 5 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain the age of first infection by ticks during the nestling stage in female chicks, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Null model | 1 | -185.69 | 373.72 | 0.00 | 0.39 |
| MHC-diversity | 2 | -184.93 | 374.59 | 0.87 | 0.25 |
| MHC-diversity ^2 | 2 | -185.58 | 375.70 | 1.99 | 0.14 |
| Hatching order | 2 | -185.72 | 375.78 | 2.06 | 0.14 |
| MHC-diversity + Hatching order | 3 | -184.94 | 376.73 | 3.01 | 0.09 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| MHC-diversity | -0.155 | 0.142 | -0.433 | 0.123 |
| MHC-diversity^2 | -0.039 | 0.124 | -0.283 | 0.204 |
| B-chick[a] | 0.011 | 0.289 | -0.556 | 0.577 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Year | 0.091 | 0.301 |

**Table S17:** a) the subset of 6 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain the age of first infection by ticks during the nestling stage in male chicks, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Null model | 0 | -214.14 | 430.26 | 0.00 | 0.37 |
| Hatching order | 1 | -213.84 | 431.67 | 1.41 | 0.18 |
| MHC-diversity | 2 | -213.76 | 431.73 | 1.47 | 0.18 |
| MHC-diversity^2 | 1 | -214.13 | 432.30 | 2.04 | 0.13 |
| MHC-diversity + Hatching order | 2 | -213.60 | 433.48 | 3.22 | 0.07 |
| MHC-diversity^2 + Hatching order | 2 | -213.84 | 433.77 | 3.51 | 0.06 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| B-chick[a] | 0.211 | 0.279 | -0.336 | 0.759 |
| MHC-diversity | -0.100 | 0.138 | -0.369 | 0.169 |
| MHC-diversity^2 | 0.006 | 0.068 | -0.128 | 0.140 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Year | 0.067 | 0.258 |

**Table S18:** a) the subset of 5 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain the maximum number of ticks carried by female chicks during the nestling stage, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. We used zero-truncated models with a negative binomial2 distribution. Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Null model | 3 | 95.91 | 198.33 | 0.00 | 0.38 |
| MHC-diversity | 4 | 95.05 | 198.95 | 0.63 | 0.28 |
| Hatching order | 4 | 95.85 | 200.54 | 2.22 | 0.13 |
| MHC-diversity^2 | 4 | 95.85 | 200.56 | 2.23 | 0.13 |
| MHC-diversity + Hatching order | 5 | 95.00 | 201.31 | 2.99 | 0.09 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.158 | 1.267 | -2.704 | 2.388 |
| MHC-diversity | -0.293 | 0.219 | -0.735 | 0.147 |
| B-chick[a] | -0.163 | 0.474 | -1.116 | 0.791 |
| MHC-diversity^2 | 0.079 | 0.234 | -0.391 | 0.550 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Year | <0.001 | <0.001 |

**Table S19:** a) the subset of 6 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain the maximum number of ticks carried by male chicks during the nestling stage, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. We used zero-truncated models with a negative binomial2 distribution. Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order | 4 | -110.08 | 228.92 | 0.00 | 0.32 |
| Null model | 3 | -111.31 | 229.06 | 0.14 | 0.30 |
| MHC-diversity + Hatching order | 5 | -110.07 | 231.30 | 2.38 | 0.10 |
| MHC-diversity^2 + Hatching order | 5 | -110.08 | 231.31 | 2.39 | 0.10 |
| MHC-diversity | 4 | -111.30 | 231.36 | 2.44 | 0.09 |
| MHC-diversity^2 | 4 | -111.31 | 231.37 | 2.45 | 0.09 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.265 | 1.952 | -4.177 | 3.646 |
| B-chick[a] | -0.808 | 0.488 | -1.787 | 0.170 |
| MHC-diversity | 0.001 | 0.256 | -0.512 | 0.514 |
| MHC-diversity^2 | -0.010 | 0.126 | -0.263 | 0.243 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Year | <0.001 | <0.001 |

**Table S20:** a) the subset of 3 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain tick loss b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Estimates different from zero are in bold. Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| MHC-diversity | 2 | -95.52 | 196.62 | 0.00 | 0.65 |
| MHC-diversity + Hatching order | 3 | -95.44 | 198.86 | 2.25 | 0.21 |
| MHC-diversity * Hatching order | 4 | -94.68 | 199.70 | 3.09 | 0.14 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| **MHC-diversity** | **0.542** | **0.203** | **0.143** | **0.940** |
| B-chick[a] | 0.040 | 0.384 | -0.712 | 0.792 |
| MHC-diversity : B-chick[a] | 0.462 | 0.353 | -0.230 | 1.154 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Year | 0.531 | 0.728 |

**Table S21:** a) the subset of 6 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 9 models considered to explain tick loss, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Null model | 0 | -101.52 | 203.06 | 0.00 | 0.31 |
| MHC-diversity^2 | 1 | -100.50 | 203.10 | 0.04 | 0.31 |
| MHC-diversity | 1 | -101.46 | 205.03 | 1.98 | 0.12 |
| Hatching order | 1 | -101.51 | 205.14 | 2.08 | 0.11 |
| MHC-diversity^2 + Hatching order | 2 | -100.50 | 205.31 | 2.25 | 0.10 |
| MHC-diversity^2 * Hatching order | 3 | -99.95 | 206.54 | 3.48 | 0.05 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| MHC-diversity^2 | 0.140 | 0.086 | -0.030 | 0.309 |
| MHC-diversity | -0.074 | 0.210 | -0.485 | 0.338 |
| B-chick[a] | -0.099 | 0.441 | -0.964 | 0.766 |
| MHC-diversity^2 : B-chick[a] | 0.394 | 0.369 | -0.329 | 1.117 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Year | <0.001 | 0.020 |

**Table S22:** a) the subset of 2 models with ΔAICc < 4 relative to the best model among the 4 models considered to explain egg volume (i.e. volume of an ellipsoid: π x (4/3) x egg width x egg length) and b) model-averaged estimates for all parameters in this subset of models. Abbreviations are described in Table S1. Estimates different from zero are in bold. In birds, offspring condition depends greatly on maternal resources allocated to the eggs (Krist, 2011). Here, B-eggs are smaller than A-eggs, and this is especially true for female eggs (Fig. S8), suggesting lower maternal investment.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order * Sex | 8 | -72139 | 1458.98 | 0.00 | 0.53 |
| Hatching order | 6 | -723.55 | 1459.23 | 0.25 | 0.47 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | 0.150 | 0.119 | -0.084 | 0.385 |
| Male[a] | -0.050 | 0.058 | -0.164 | 0.063 |
| **B-chick[b]** | **-0.569** | **0.066** | **-0.698** | **-0.440** |
| **B-chick[b] : Male[a]** | **0.168** | **0.084** | **0.003** | **0.333** |

[a] Relative to females
[b] Relative to first-hatched A-chicks

**Table S23:** a) the subset of 2 models with ΔAICc < 4 relative to the best model among the 4 models considered to explain chick body condition at 15 days old and b) model-averaged estimates for all parameters in this subset of models. Chick size was included in all models. Abbreviations are described in Table S1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Sex | 6 | -264.63 | 541.57 | 0.00 | 0.85 |
| Sex * Hatching order | 8 | -264.29 | 545.11 | 3.54 | 0.15 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.220 | 0.108 | -0.433 | -0.007 |
| **Male[a]** | **0.196** | **0.076** | **0.046** | **0.346** |
| **Size** | **0.675** | **0.043** | **0.589** | **0.760** |
| B-chick[b] | -0.092 | 0.110 | -0.308 | 0.124 |
| B-chick[b] : Male[a] | 0.084 | 0.147 | -0.206 | 0.374 |

[a] Relative to females

[b] Relative to first-hatched A-chicks

**Table S24:** a) the subset of 4 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 5 models considered to explain female mortality, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. We restricted this analysis to 1-chick broods (i.e. one egg did not hatch, thus preventing competition and aggression between siblings; n = 77 female chicks). Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Null model | 4 | -96.92 | 203.86 | 0.00 | 0.52 |
| Hatching order | 5 | -96.64 | 205.71 | 1.85 | 0.20 |
| MHC-diversity | 5 | -96.75 | 205.78 | 1.92 | 0.20 |
| MHC-diversity + Hatching order | 6 | -96.32 | 207.53 | 3.67 | 0.08 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| B-chick[a] | 0.258 | 0.510 | -0.742 | 1.257 |
| MHC-diversity | -0.130 | 0.197 | -0.517 | 0.256 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.643 | 0.802 |
| Year | <0.001 | 0.020 |

**Table S25:** a) the subset of 4 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 5 models considered to explain female body mass growth rate over the first 10 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. We restricted this analysis to 1-chick broods (i.e. one egg did not hatch, thus preventing competition and aggression between siblings; n = 34 female chicks). Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| MHC-diversity | 4 | -41.83 | 93.05 | 0.00 | 0.42 |
| Null model | 3 | -43.27 | 93.35 | 0.30 | 0.36 |
| MHC-diversity + Hatching order | 5 | -41.75 | 95.64 | 2.59 | 0.11 |
| Hatching order | 4 | -43.22 | 95.83 | 2.78 | 0.10 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.223 | 0.335 | -0.908 | 0.461 |
| MHC-diversity | 0.215 | 0.123 | -0.037 | 0.466 |
| B-chick[a] | -0.102 | 0.272 | -0.659 | 0.455 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Year | 0.650 | 0.806 |

**Table S26:** a) the subset of 4 models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 5 models considered to explain female body size growth rate over the first 10 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. We restricted this analysis to 1-chick broods (i.e. one egg did not hatch, thus preventing competition and aggression between siblings; n = 34 female chicks). Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| MHC-diversity | 4 | -41.83 | 95.81 | 0.00 | 0.41 |
| Null model | 3 | -43.27 | 96.11 | 0.31 | 0.35 |
| MHC-diversity + Hatching order | 5 | -41.75 | 98.20 | 2.40 | 0.12 |
| Hatching order | 4 | -43.22 | 98.47 | 2.66 | 0.11 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.148 | 0.310 | -0.780 | 0.484 |
| MHC-diversity | 0.232 | 0.133 | -0.039 | 0.503 |
| B-chick[a] | -0.161 | 0.290 | -0.753 | 0.432 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Year | 0.492 | 0.701 |

**Table S27:** a) the models including functional MHC-diversity, with ΔAICc < 4 relative to the best model among the 5 models considered to explain the number of consecutive days female chicks were observed with ticks (i.e. the time to clear tick infection), b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. We restricted this analysis to 1-chick broods (i.e. one egg did not hatch, thus preventing competition and aggression between siblings; n = 14 female chicks). Estimates different from zero are in bold. Abbreviations are described in Table S1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| MHC-diversity | 1 | -19.95 | 42.23 | 0.00 | 0.39 |
| MHC-diversity + Hatching order | 2 | -19.00 | 43.09 | 0.86 | 0.35 |
| Null model | 0 | -21.56 | 43.67 | 1.44 | 0.19 |
| Hatching order | 1 | -20.71 | 44.57 | 2.34 | 0.12 |
| MHC-diversity * Hatching order | 3 | -18.91 | 46.22 | 3.99 | 0.05 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| MHC-diversity | 0.635 | 0.372 | -0.093 | 1.364 |
| B-chick[a] | 0.945 | 0.683 | -0.394 | 2.284 |
| MHC-diversity : B-chick[a] | 0.287 | 0.673 | -1.031 | 1.606 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Year | <0.001 | 0.20 |

**Supplementary analyses: Number of MHC-II alleles and MHC-II divergence**

**Materials and methods**

*MHC measures*

In addition to Faith's MHC-II diversity, we also estimated MHC-diversity as the number of functional MHC-II alleles and the MHC-II divergence for each chick. We calculated the number of MHC alleles as the number of amino acid PBR sequences per individuals. To calculate MHC-II divergence, we followed the approach of Schwensow et al. (2007). We first described the chemical binding properties of each amino acid in the PBRs with the Sandberg's five physico-chemical descriptors (z-descriptors; Sandberg, Eriksson, Jonsson, Sjostrom, & Wold, 1998). Then, using the resulting matrix, we computed the Euclidean distance between all possible pairs of functional alleles (Lenz, Wells, Pfeiffer, & Sommer, 2009), using the R function "distance" in the philentropy R package (Drost, 2018). We calculated MHC-II divergence as the sum of Euclidean distances between each pair of alleles possessed by an individual, divided by the number of allele pairs. It was thus not possible to calculate MHC-II divergence for chicks carrying only one MHC-II allele (n = 2 individuals). Chick functional MHC-II divergence varied from 6.57 to 21.93 (mean ± s.d.: 17.32 ± 1.79; Figure S11) and did not significantly vary among years (Kruskal–Wallis, U = 10.22, df = 8, p = 0.24; Figure S12). MHC-divergence is theoretically not related to the number of alleles but, in our dataset, there was a negative correlation between these measures (Pearson correlation, t = -5.67; r = 0.21; p < 0.001; n = 697, as in Roved 2019). Including both measures in the same models leads to VIF values > 5, indicating collinearity issues (Zuur et al. 2010). We therefore did separate analyses for these two measures.

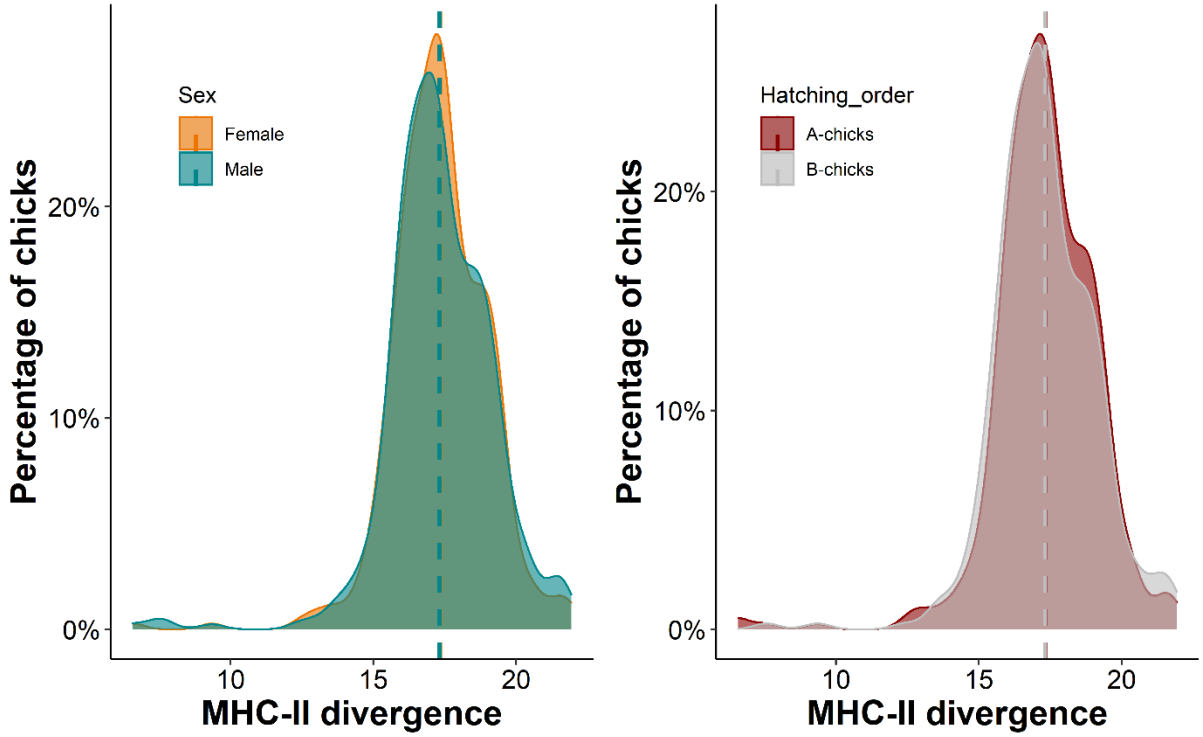


**Figure S11.** Distribution of functional MHC-II divergence in chicks according to sex (left figure) with females in orange and males in blue, and hatching order (right figure) with A-chicks in pink and B-chicks in grey. Dashed lines represent mean MHC-II divergence.

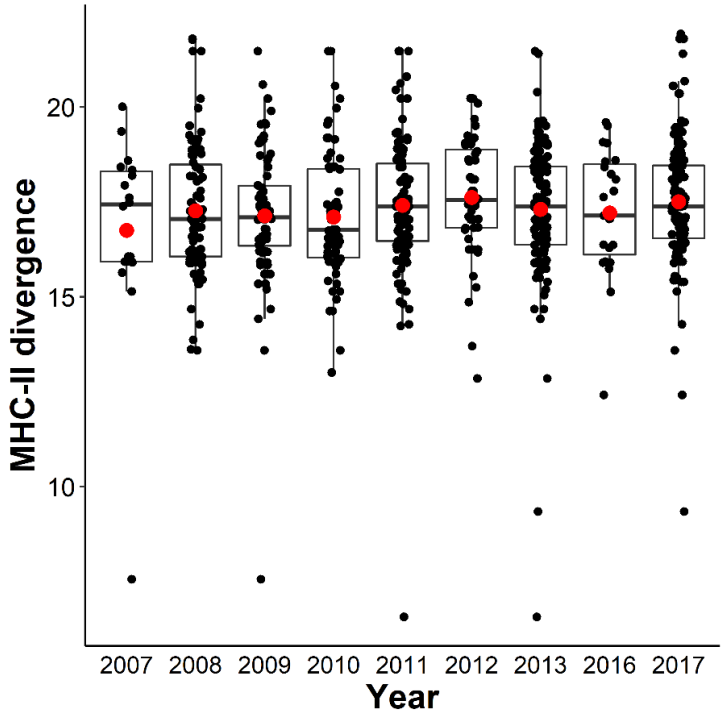


**Figure S12.** Boxplots of MHC-II divergence of chicks according to year. Red dots represent mean MHC-II divergence.

*Statistical analyses*

We used the same statistical approach as for MHC-II diversity (see main manuscript). For each fitness-related trait, we built a set of models that included either number of MHC-II alleles or MHC-II divergence. We also included the square of the MHC-II measure, sex, hatching order and two- and three-way interactions between sex, hatching order and the MHC-II variable. Because most chicks had 3 or 4 alleles in the restricted dataset used for tick analyses, we transformed the "number of MHC-II alleles" in a binary variable with number of MHC-II alleles $\leq 3$ or $\geq 4$. The square of number of MHC-II alleles and the interactions that included this variable were thus removed from the models.

**RESULTS**

*Number of MHC-II alleles*

The age of first infection by ticks at the nest stage was significantly associated with the interaction between the number of MHC-II alleles and sex (estimate ± s.e. = 1.02 ± 0.46; 95% CI: 0.13, 1.92; Table S28). Females with three MHC-II alleles or less were infected more rapidly and were more likely to be infected than females with four MHC-II alleles or more (Figure S13). However, the number of functional MHC-II alleles was not significantly associated with survival (Table S29), nor with body condition or body size at hatching (Tables S30, S31), nor with body mass or size growth rates (Tables S32-S35), nor with the probability to lose all ticks (Table S36) or with the maximum number of ticks (Table S37).

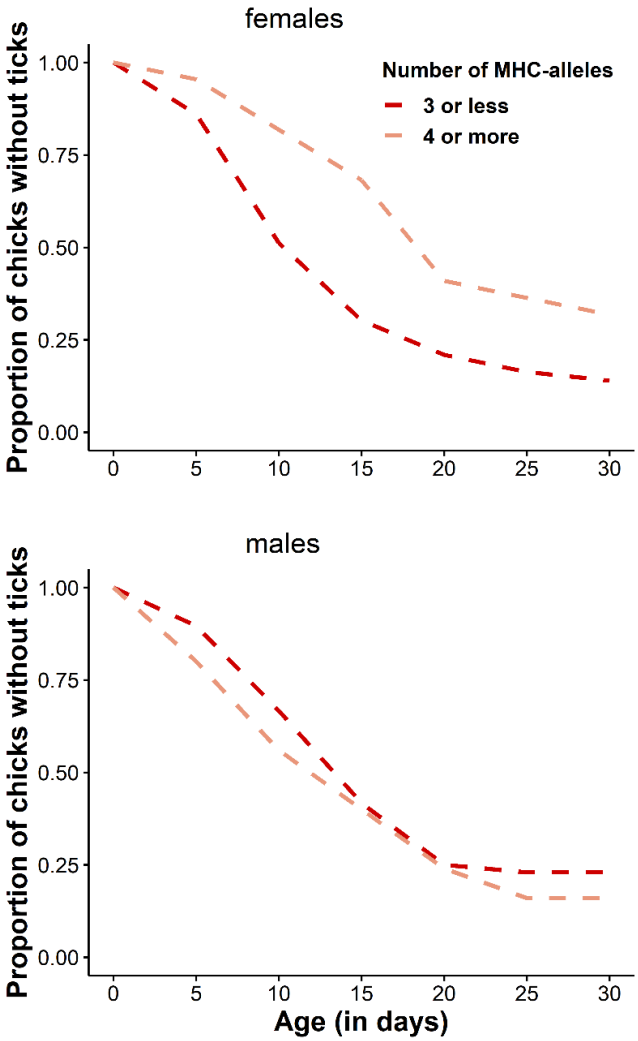


**Figure S13.** Proportion of chicks without ticks according to age and number of functional MHC-II alleles for females (n = 65) and males (n = 73). Because most chicks had 3 or 4 alleles in this restricted dataset used for tick analyses, we recast the "number of MHC-II alleles" as a binary variable with number of MHC-II alleles ≤ 3 (in red) or ≥ 4 (in pink).

*MHC-II divergence*

There was a significant effect of the interaction between the square of MHC-II divergence and sex on body condition at hatching (estimate ± s.e. = 0.05 ± 0.02, 95% CI: 0.002, 0.09; Table S38) and on body mass growth rate over the first 10 days (estimate ± s.e. = 0.13 ± 0.06, 95% CI: 0.012, 0.247; Table S39), and between MHC-II divergence and hatching order on body size at hatching (estimate ± s.e. = 0.15 ± 0.07, 95% CI: 0.014, 0.288; Table S40). However, all these interactions became non-significant after removing one A-male with very low MHC-II divergence and high morphological or growth values (Tables S41-S43). In the body mass growth rate analysis, the square of MHC-II divergence became significant after removing this

male (estimate ± s.e. = -0.098 ± 0.045, 95% CI: -0.188, -0.009; Table S42; Figure S14). Chicks with intermediate MHC-II divergence grew faster than those with low or high MHC-II divergence. MHC-II divergence was not significantly associated with survival (Table S44), nor with tick infection (Tables S45-S47), nor with other growth measures (Tables S48-S50).

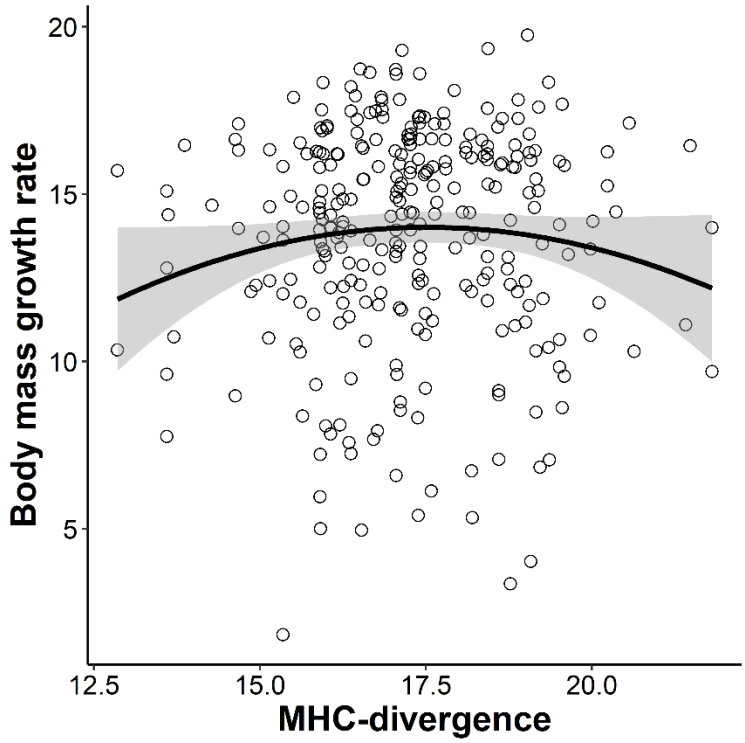


**Figure S14.** Growth rate of chick body mass over the first 10 days according to functional MHC-II divergence. Size growth rate was calculated as the maximum slope of a logistic growth curve between morphological measures and age (see the main manuscript for more details). Regression lines were derived from a model including MHC-II divergence of chicks and its square as fixed effects. Random effects (year and pair ID) were not considered in the models used for graphic representations. Shaded areas represent confidence intervals.

**Table S28:** a) the subset of 5 models including the number of MHC alleles, with ΔAICc < 4 relative to the best model among the 11 models considered to explain the age of first infection by ticks during the nestling stage in chicks, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. The clutch ID random effect was removed from models because associated variance estimates were virtually zero. Because most chicks had 3 or 4 alleles in this restricted dataset used for tick analyses, we transformed the "number of MHC alleles" to a binary variable with number of MHC alleles ≤ 3 or ≥ 4. The square of number of MHC alleles and the interactions that included this variable were thus removed from the models. Abbreviations are described in Table S1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Number of MHC alleles * Sex | 20 | -461.47 | 971.62 | 0.00 | 0.46 |
| Null model | 18 | -464.24 | 972.47 | 0.85 | 0.30 |
| Sex | 19 | -464.20 | 974.85 | 3.23 | 0.09 |
| Number of MHC alleles | 20 | -462.53 | 974.95 | 3.33 | 0.09 |
| Hatching order | 20 | -463.68 | 975.60 | 3.98 | 0.06 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Number of MHC alleles | -0.743 | 0.382 | -1.491 | 0.004 |
| Male[a] | -0.294 | 0.277 | -0.838 | 0.249 |
| **Number of MHC alleles : Male[a]** | **1.023** | **0.458** | **0.125** | **1.921** |
| B-chick[b] | 0.135 | 0.213 | -0.282 | 0.552 |

[a] Relative to females

[b] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Pair | 0.115 | 0.339 |
| Year | 0.209 | 0.457 |

**Table S29:** a) the subset of 4 models including the number of MHC alleles, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick mortality, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order | 38 | -750.15 | 1586.08 | 0.00 | 0.46 |
| Number of MHC alleles + Hatching order | 39 | -750.43 | 1587.61 | 1.53 | 0.22 |
| Number of MHC alleles^2 + Hatching order | 39 | -750.31 | 1587.88 | 1.81 | 0.19 |
| Number of MHC alleles * Hatching order | 39 | -750.76 | 1588.58 | 2.51 | 0.13 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| **B-chick[a]** | **0.914** | **0.182** | **0.556** | **1.271** |
| Number of MHC alleles | 0.036 | 0.122 | -0.203 | 0.276 |
| Number of MHC alleles^2 | 0.006 | 0.067 | -0.124 | 0.137 |
| Number of MHC alleles: B-chick[a] | -0.111 | 0.183 | -0.470 | 0.249 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.001 | 0.020 |
| Pair | 0.290 | 0.538 |
| Year | 1.701 | 1.304 |

**Table S30:** a) the subset of 9 models including the number of MHC alleles, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body condition at hatching, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Chick size was included in all models. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Number of MHC alleles^2 + Hatching order + Sex | 9 | -805.68 | 1629.64 | 0.00 | 0.21 |
| Hatching order | 7 | -807.84 | 1629.84 | 0.21 | 0.19 |
| Number of MHC alleles + Hatching order + Sex | 9 | -805.98 | 1630.23 | 0.59 | 0.16 |
| Number of MHC alleles^2 + Hatching order | 8 | -807.28 | 1630.78 | 1.15 | 0.12 |
| Number of MHC alleles + Hatching order | 8 | -807.57 | 1631.35 | 1.71 | 0.09 |
| Number of MHC alleles * Hatching order | 9 | -806.63 | 1631.53 | 1.89 | 0.08 |
| Number of MHC alleles^2 + Hatching order + Sex + all two-way interactions | 12 | -803.74 | 1631.94 | 2.30 | 0.07 |
| Number of MHC alleles * Hatching order | 9 | -807.39 | 1633.05 | 3.41 | 0.04 |
| Number of MHC alleles + Hatching order + Sex + two-way interactions | 12 | -804.58 | 1633.63 | 4.00 | 0.03 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | 0.054 | 0.135 | -0.205 | 0.313 |
| Number of MHC alleles^2 | 0.021 | 0.021 | -0.017 | 0.059 |
| Male[b] | 0.081 | 0.075 | -0.064 | 0.227 |
| **B-chick[a]** | **-0.441** | **0.060** | **-0.560** | **-0.324** |
| **Size** | **0.258** | **0.032** | **0.196** | **0.320** |
| Number of MHC alleles | -0.019 | 0.034 | -0.088 | 0.051 |
| Number of MHC alleles^2 : B-chick[a] | -0.035 | 0.036 | -0.098 | 0.028 |
| B-chick[a] : Male[b] | 0.182 | 0.112 | -0.038 | 0.402 |
| Number of MHC alleles^2 : Male[b] | 0.016 | 0.033 | -0.050 | 0.081 |
| Number of MHC alleles : B-chick[a] | -0.028 | 0.054 | -0.134 | 0.079 |
| Number of MHC alleles : Male[b] | -0.017 | 0.057 | -0.130 | 0.095 |

[a] Relative to first-hatched A-chicks

[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.070 | 0.265 |
| Pair | 0.309 | 0.556 |
| Year | 0.119 | 0.345 |

**Table S31:** a) the subset of 13 models including the number of MHC alleles, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body size at hatching, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table 1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Number of MHC alleles + Hatching order | 7 | -924.51 | 1863.20 | 0.00 | 0.17 |
| Hatching order | 6 | -925.62 | 1863.36 | 0.16 | 0.15 |
| Number of MHC alleles | 6 | -925.95 | 1864.03 | 0.83 | 0.11 |
| Null model | 5 | -926.98 | 1864.04 | 0.85 | 0.11 |
| Number of MHC alleles + Hatching order + Sex | 8 | -924.17 | 1864.56 | 1.36 | 0.08 |
| Number of MHC alleles * Hatching order | 8 | -924.47 | 1865.15 | 1.96 | 0.06 |
| Number of MHC alleles^2 + Hatching order | 7 | -925.58 | 1865.32 | 2.13 | 0.06 |
| Sex | 6 | -926.61 | 1865.35 | 2.16 | 0.06 |
| Number of MHC alleles + Sex | 7 | -925.64 | 1865.44 | 2.24 | 0.05 |
| Number of MHC alleles * Sex | 8 | -924.75 | 1865.72 | 2.52 | 0.05 |
| Number of MHC alleles^2 | 6 | -926.94 | 1866.01 | 2.81 | 0.04 |
| Number of MHC alleles^2 + Sex | 8 | -925.19 | 1866.60 | 3.40 | 0.03 |
| Number of MHC alleles^2 + Hatching order | 8 | -925.43 | 1867.07 | 3.88 | 0.02 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | 0.072 | 0.134 | -0.191 | 0.336 |
| Number of MHC alleles | -0.051 | 0.044 | -0.136 | 0.035 |
| B-chick[a] | -0.110 | 0.067 | -0.240 | 0.021 |
| Male[b] | 0.059 | 0.071 | -0.080 | 0.198 |
| Number of MHC alleles : B-chick[a] | 0.021 | 0.069 | -0.114 | 0.156 |
| Number of MHC alleles^2 | 0.007 | 0.022 | -0.036 | 0.050 |
| Number of MHC alleles : Male[b] | -0.095 | 0.071 | -0.234 | 0.045 |
| Number of MHC alleles^2 : B-chick[a] | -0.022 | 0.041 | -0.102 | 0.058 |

[a] Relative to first-hatched A-chicks
[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.000 | 0.000 |
| Pair | 0.243 | 0.493 |
| Year | 0.123 | 0.351 |

**Table S32:** a) the subset of 6 models including the number of MHC alleles, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body mass growth rate over the first 10 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Number of MHC alleles^2 + Hatching order + Sex | 8 | -356.71 | 729.93 | 0.00 | 0.38 |
| Hatching order | 6 | -359.45 | 731.19 | 1.26 | 0.20 |
| Number of MHC alleles + Hatching order + Sex | 8 | -357.45 | 731.40 | 1.47 | 0.18 |
| Number of MHC alleles^2 + Hatching order | 7 | -359.07 | 732.53 | 2.60 | 0.10 |
| Number of MHC alleles + Hatching order | 7 | -359.43 | 733.26 | 3.33 | 0.07 |
| Sex | 6 | -360.59 | 733.48 | 3.55 | 0.06 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.319 | 0.261 | -0.832 | 0.194 |
| Number of MHC alleles^2 | -0.037 | 0.032 | -0.101 | 0.027 |
| **Male[b]** | **0.195** | **0.092** | **0.013** | **0.376** |
| **B-chick[a]** | **-0.231** | **0.090** | **-0.408** | **-0.055** |
| Number of MHC alleles | 0.006 | 0.048 | -0.088 | 0.100 |

[a] Relative to first-hatched A-chicks
[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.000 | 0.000 |
| Pair | 0.164 | 0.405 |
| Year | 0.457 | 0.676 |

**Table S33:** a) the subset of 7 models including the number of MHC alleles, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body size growth rate over the first 10 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order | 6 | -363.29 | 738.87 | 0.00 | 0.37 |
| Number of MHC alleles^2 + Hatching order | 7 | -363.09 | 740.58 | 1.72 | 0.16 |
| Number of MHC alleles^2 + Hatching order + Sex | 8 | -362.21 | 740.93 | 2.06 | 0.13 |
| Number of MHC alleles + Hatching order | 7 | -363.28 | 740.96 | 2.10 | 0.13 |
| Number of MHC alleles + Hatching order + Sex | 8 | -362.55 | 741.60 | 2.74 | 0.09 |
| Number of MHC alleles^2 * Hatching order | 8 | -362.99 | 742.49 | 3.63 | 0.06 |
| Number of MHC alleles * Hatching order | 8 | -363.00 | 742.51 | 3.65 | 0.06 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.224 | 0.237 | -0.701 | 0.233 |
| **B-chick[a]** | **-0329** | **0.083** | **-0.492** | **-0.167** |
| Number of MHC alleles^2 | -0.024 | 0.034 | -0.091 | 0.042 |
| Male[b] | 0.116 | 0.089 | -0.060 | 0.292 |
| Number of MHC | -0.004 | 0.052 | -0.105 | 0.097 |
| Number of MHC alleles : B-chick[a] | 0.066 | 0.088 | -0.106 | 0.239 |
| Number of MHC alleles^2 : B-chick[a] | 0.024 | 0.052 | -0.079 | 0.127 |

[a] Relative to first-hatched A-chicks
[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.345 | 0.588 |
| Pair | 0.063 | 0.250 |
| Year | 0.432 | 0.657 |

**Table S34:** a) the subset of 5 models including the number of MHC alleles, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body mass growth rate over 35 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Number of MHC alleles + Hatching order + Sex | 8 | -275.29 | 567.30 | 0.00 | 0.53 |
| Number of MHC alleles + Sex | 7 | -277.42 | 569.39 | 2.09 | 0.19 |
| Number of MHC alleles^2 + Hatching order + Sex | 8 | -276.96 | 570.64 | 3.34 | 0.10 |
| Sex | 6 | -279.17 | 570.75 | 3.45 | 0.09 |
| Number of MHC alleles * Sex | 8 | -277.08 | 570.87 | 3.58 | 0.09 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.416 | 0.198 | -0.805 | -0.026 |
| Number of MHC alleles | 0.108 | 0.072 | -0.034 | 0.250 |
| **B-chick[a]** | **-0.263** | **0.119** | **-0.498** | **-0.028** |
| **Male[b]** | **0.504** | **0.122** | **0.264** | **0.744** |
| Number of MHC alleles^2 | -0.018 | 0.043 | -0.103 | 0.067 |
| Number of MHC alleles : Male[b] | 0.114 | 0.135 | -0.152 | 0.380 |

[a] Relative to first-hatched A-chicks

[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.119 | 0.345 |
| Pair | 0.118 | 0.343 |
| Year | 0.223 | 0.472 |

**Table S35:** a) the subset of 2 models including the number of MHC alleles, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body size growth rate over 35 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Number of MHC alleles^2 + Hatching order + Sex | 8 | -253.39 | 523.51 | 0.00 | 0.50 |
| Number of MHC alleles + Hatching order + Sex | 8 | -253.74 | 524.20 | 0.69 | 0.36 |
| Number of MHC alleles + Hatching order + Sex + two-way interactions | 11 | -251.38 | 526.11 | 2.60 | 0.14 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.311 | 0.250 | -0.807 | 0.185 |
| Number of MHC alleles^2 | -0.032 | 0.039 | -0.109 | 0.044 |
| **B-chick[a]** | **-0.378** | **0.126** | **-0.627** | **-0.129** |
| **Male[b]** | **0.410** | **0.118** | **0.177** | **0.643** |
| Number of MHC alleles | -0.006 | 0.076 | -0.156 | 0.144 |
| Male[b] : B-chick[a] | 0.250 | 0.228 | -0.199 | 0.699 |
| Number of MHC alleles : Male[b] | -0.076 | 0.121 | -0.315 | 0.164 |
| Number of MHC alleles : B-chick[a] | 0.187 | 0.110 | -0.029 | 0.403 |

[a] Relative to first-hatched A-chicks
[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.208 | 0.456 |
| Pair | 0.000 | 0.000 |
| Year | 0.438 | 0.662 |

**Table S36:** a) the subset of 4 models including the number of MHC alleles, with ΔAICc < 4 relative to the best model among the 11 models considered to explain tick loss, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. The clutch ID random effect was removed from models because associated variance estimates were virtually zero. Because most chicks had 3 or 4 alleles in this restricted dataset used for tick analyses, we transformed the "number of MHC alleles" to a binary variable with number of MHC alleles ≤ 3 or ≥ 4. The square of number of MHC alleles and the interactions that included this variable were thus removed from the models. Abbreviations are described in Table 1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Null model | 24 | -231.32 | 532.71 | 0.00 | 0.60 |
| Sex | 25 | -231.35 | 535.67 | 296 | 0.14 |
| Number of MHC alleles | 25 | -231.28 | 535.74 | 3.03 | 0.13 |
| Hatching order | 25 | -231.30 | 535.79 | 3.08 | 0.13 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Male[a] | 0.027 | 0.299 | -0.560 | 0.614 |
| Number of MHC alleles | 0.152 | 0.330 | -0.631 | 0.662 |
| B-chick[b] | 0.059 | 0.307 | -0.543 | 0.661 |

[b] Relative to females

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Pair | 0.144 | 0.379 |
| Year | 0.662 | 0.814 |

**Table S37:** a) the subset of 4 models including the number of MHC alleles, with ΔAICc < 4 relative to the best model among the 11 models considered to explain the maximum number of ticks carried by chicks during the nestling stage, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. The clutch ID random effect was removed from models because associated variance estimates were virtually zero. We used zero-truncated models with a Poisson distribution. Because most chicks had 3 or 4 alleles in this restricted dataset used for tick analyses, we transformed the "number of MHC alleles" to a binary variable with number of MHC alleles ≤ 3 or ≥ 4. The square of number of MHC alleles and the interactions that included this variable were thus removed from the models. Abbreviations are described in Table 1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Null model | 3 | -208.16 | 422.56 | 0.00 | 0.49 |
| Hatching order | 4 | -208.07 | 424.53 | 1.98 | 0.18 |
| Sex | 4 | -208.15 | 424.67 | 2.12 | 0.17 |
| Number of MHC alleles | 4 | -208.16 | 424.71 | 2.15 | 0.17 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | 0.636 | 0.322 | -0.002 | 1.275 |
| B-chick[a] | -0.080 | 0.188 | -0.450 | 0.292 |
| Male[b] | -0.041 | 0.212 | -0.461 | 0.379 |
| Number of MHC alleles | -0.010 | 0.234 | -0.475 | 0.454 |

[a] Relative to first-hatched A-chicks

[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Pair | 0.200 | 0.448 |
| Year | 1.266 | 1.125 |

**Table S38:** a) the subset of 2 models including MHC-II divergence, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body condition at hatching, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Chick size was included in all models. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order + MHC-divergence^2 + Sex + two-way interactions | 12 | -796.46 | 1617.40 | 0.00 | 0.73 |
| Hatching order + MHC-divergence^2 + Sex + two-way interactions + three-way interaction | 13 | -796.40 | 1619.35 | 1.95 | 0.27 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | 0.050 | 0.135 | -0.215 | 0.315 |
| MHC-divergence^2 | 0.011 | 0.020 | -0.029 | 0.050 |
| **B-chick[a]** | **-0.503** | **0.081** | **-0.662** | **-0.344** |
| Male[b] | -0.038 | 0.079 | -0.019 | 0.116 |
| **Size** | **0.252** | **0.031** | **0.190** | **0.313** |
| B-chick[a] : Male[b] | 0.204 | 0.114 | -0.020 | 0.427 |
| **MHC-divergence^2 : Male[b]** | **0.048** | **0.024** | **0.002** | **0.094** |
| MHC-divergence^2 : B-chick[a] | -0.035 | 0.027 | -0.088 | 0.018 |
| MHC-divergence^2 : B-chick[a] : Male[b] | -0.016 | 0.045 | -0.105 | 0.073 |

[a] Relative to first-hatched A-chicks

[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.060 | 0.245 |
| Pair | 0.289 | 0.536 |
| Year | 0.123 | 0.351 |

**Table S39:** a) the subset of 9 models including MHC-II divergence, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body mass growth rate over the first 10 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. The clutch ID random effect was removed from models because associated variance estimates were virtually zero. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order + MHC-divergence^2 + Sex | 7 | -356.85 | 728.10 | 0.00 | 0.25 |
| Hatching order | 5 | -359.45 | 729.11 | 1.00 | 0.15 |
| Hatching order+ MHC-divergence + Sex | 7 | -357.45 | 729.30 | 1.19 | 0.14 |
| MHC-divergence^2 * Sex | 7 | -357.52 | 729.43 | 1.33 | 0.13 |
| Hatching order + MHC-divergence^2 + Sex + two-way interactions | 10 | -354.59 | 729.97 | 1.87 | 0.10 |
| Hatching order + MHC-divergence^2 | 6 | -358.94 | 730.18 | 2.08 | 0.09 |
| Hatching order + MHC-divergence | 6 | -359.45 | 731.19 | 3.09 | 0.05 |
| Sex | 5 | -360.59 | 731.39 | 3.29 | 0.05 |
| Hatching order * MHC-divergence^2 | 7 | -358.77 | 731.93 | 3.83 | 0.04 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.308 | 0.261 | -0.821 | 0.206 |
| MHC-divergence^2 | -0.063 | 0.068 | -0.197 | 0.070 |
| **B-chick[a]** | **0.234** | **0.100** | **-0.431** | **-0.037** |
| Male[b] | -0.146 | 0.115 | -0.080 | 0.373 |
| MHC-divergence | -0.002 | 0.481 | -0.097 | 0.092 |
| **MHC-divergence^2 : Male[b]** | **0.129** | **0.060** | **0.012** | **0.247** |
| B-chick[a] : Male[b] | 0.014 | 0.188 | -0.356 | 0.383 |
| MHC-divergence^2 : B-chick[a] | 0.019 | 0.068 | -0.114 | 0.152 |

[a] Relative to first-hatched A-chicks

[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Pair | 0.210 | 0.458 |
| Year | 0.521 | 0.722 |

**Table S40:** a) the subset of 13 models including MHC-II divergence, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body size at hatching, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. The clutch ID random effect was removed from models because associated variance estimates were virtually zero. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order * MHC-divergence | 7 | -921.73 | 1857.62 | 0.00 | 0.18 |
| Hatching order | 5 | -924.11 | 1858.31 | 0.69 | 0.13 |
| Hatching order + MHC-divergence^2 | 6 | -923.14 | 1858.41 | 0.78 | 0.12 |
| Null model | 4 | -925.40 | 1858.87 | 1.24 | 0.09 |
| MHC-divergence^2 | 5 | -924.42 | 1858.93 | 1.30 | 0.09 |
| MHC-divergence^2 * Sex | 7 | -922.52 | 1859.21 | 1.59 | 0.08 |
| Hatching order + Sex + MHC-divergence^2 | 7 | -922.78 | 1859.73 | 2.10 | 0.06 |
| Sex | 5 | -925.04 | 1860.16 | 2.54 | 0.05 |
| Hatching order + MHC-divergence | 6 | -924.06 | 1860.24 | 2.62 | 0.05 |
| Sex + MHC-divergence^2 | 6 | -924.08 | 1860.29 | 2.67 | 0.05 |
| Hatching order * MHC-divergence^2 | 7 | -923.08 | 1860.32 | 2.70 | 0.05 |
| MHC-divergence | 5 | -925.35 | 1860.79 | 3.17 | 0.04 |
| Hatching order + Sex + MHC-divergence | 7 | -923.67 | 1861.50 | 3.88 | 0.03 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | 0.065 | 0.134 | -0.199 | 0.328 |
| MHC-divergence | -0.028 | 0.055 | -0.135 | 0.079 |
| B-chick[a] | -0.106 | 0.067 | -0.237 | 0.025 |
| **B-chick[a] : MHC-divergence** | **0.151** | **0.070** | **0.014** | **0.288** |
| MHC-divergence^2 | 0.012 | 0.019 | -0.024 | 0.049 |
| Male[b] | 0.047 | 0.075 | -0.101 | 0.194 |
| MHC-divergence^2 : Male[b] | 0.046 | 0.026 | -0.005 | 0.097 |
| MHC-divergence^2 : B-chick[a] | -0.010 | 0.027 | -0.063 | 0.044 |

[a] Relative to first-hatched A-chicks
[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Pair | 0.240 | 0.490 |
| Year | 0.119 | 0.346 |

**Table S41:** a) the subset of 3 models including MHC-II divergence, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body condition at hatching, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Chick size was included in all models. One A-male with low MHC-divergence and high body condition was removed from this analysis. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order * MHC-divergence^2 | 9 | -362.51 | 743.66 | 0.00 | 0.64 |
| Hatching order + MHC-divergence^2 + Sex + two-way interactions + three-way interaction | 13 | -359.26 | 745.82 | 2.16 | 0.22 |
| Hatching order + MHC-divergence^2 + Sex + two-way interactions | 12 | -360.73 | 746.58 | 2.91 | 0.15 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | 0.065 | 0.159 | -0.378 | 0.247 |
| MHC-divergence^2 | 0.020 | 0.074 | -0.126 | 0.166 |
| B-chick[a] | -0.284 | 0.185 | -0.648 | 0.079 |
| Male[b] | 0.143 | 0.137 | -0.412 | 0.127 |
| **Size** | **0.374** | **0.052** | **0.272** | **0.476** |
| B-chick[a] : Male[b] | 0.407 | 0.234 | -0.055 | 0.868 |
| MHC-divergence^2 : Male[b] | 0.115 | 0.087 | -0.057 | 0.287 |
| MHC-divergence^2 : B-chick[a] | -0.098 | 0.102 | -0.298 | 0.102 |
| MHC-divergence^2 : B-chick[a] : Male[b] | -0.232 | 0.134 | -0.494 | 0.030 |

[a] Relative to first-hatched A-chicks
[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.000 | 0.000 |
| Pair | 0.278 | 0.527 |
| Year | 0.119 | 0.345 |

**Table S42:** a) the subset of 5 models including MHC-II divergence, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body mass growth rate over the first 10 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. The clutch ID random effect was removed from models because associated variance estimates were virtually zero. One A-male with low MHC-divergence and high body mass growth rate was removed from this analysis. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order + MHC-divergence^2 + Sex | 7 | -352.53 | 719.45 | 0.00 | 0.50 |
| Hatching order + MHC-divergence^2 | 6 | -354.62 | 721.53 | 2.08 | 0.18 |
| Hatching order * MHC-divergence | 7 | -353.77 | 721.95 | 2.49 | 0.14 |
| Hatching order + MHC-divergence^2 + Sex + two-way interactions | 10 | -350.87 | 722.54 | 3.08 | 0.11 |
| Sex + MHC-divergence^2 | 6 | -355.53 | 723.36 | 3.91 | 0.07 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.253 | 0.266 | -0.776 | 0.206 |
| **MHC-divergence^2** | **-0.098** | **0.045** | **-0.188** | **-0.009** |
| **B-chick[a]** | **-0.248** | **0.109** | **-0.463** | **-0.034** |
| Male[b] | 0.178 | 0.985 | -0.016 | 0.371 |
| MHC-divergence^2 : B-chick[a] | 0.089 | 0.062 | -0.032 | 0.211 |
| B-chick[a] : Male[b] | 0.017 | 0.185 | 0.348 | 0.381 |
| MHC-divergence^2 : Male[b] | 0.056 | 0.059 | 0.060 | 0.173 |

[a] Relative to first-hatched A-chicks
[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Pair | 0.216 | 0.464 |
| Year | 0.522 | 0.722 |

**Table S43:** a) the subset of 13 models including MHC-II divergence, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body size at hatching, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. The clutch ID random effect was removed from models because associated variance estimates were virtually zero. One A-male with low MHC-divergence and high body size was removed from this analysis. Abbreviations are described in Table 1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order | 5 | -922.24 | 1854.58 | 0.00 | 0.17 |
| Null model | 4 | -923.43 | 1854.93 | 0.35 | 0.14 |
| Hatching order * MHC-divergence | 7 | -920.40 | 1854.97 | 0.39 | 0.14 |
| Hatching order + MHC-divergence | 6 | -921.80 | 1855.72 | 1.15 | 0.10 |
| MHC-divergence | 5 | -922.98 | 1856.05 | 1.47 | 0.08 |
| Sex | 5 | -923.14 | 1856.37 | 1.79 | 0.07 |
| Hatching order + MHC-divergence^2 | 6 | -922.21 | 1856.54 | 1.96 | 0.06 |
| MHC-divergence^2 | 5 | -923.40 | 1856.89 | 2.31 | 0.05 |
| Hatching order + MHC-divergence + Sex | 7 | -921.49 | 1857.14 | 2.57 | 0.05 |
| Sex + MHC-divergence | 6 | -922.69 | 1857.51 | 2.93 | 0.04 |
| Hatching order + MHC-divergence^2 + Sex | 7 | -921.89 | 1857.95 | 3.37 | 0.03 |
| MHC-divergence^2 + Sex | 6 | -923.11 | 1858.34 | 3.77 | 0.03 |
| Hatching order * MHC-divergence^2 | 7 | -922.14 | 1858.46 | 3.88 | 0.02 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | 0.071 | 0.134 | -0.192 | 0.334 |
| B-chick[a] | -0.103 | 0.066 | -0.233 | 0.028 |
| MHC-divergence | 0.017 | 0.049 | -0.079 | 0.114 |
| B-chick[a] : MHC-divergence | 0.116 | 0.069 | -0.020 | 0.253 |
| Male[b] | 0.055 | 0.071 | -0.085 | 0.195 |
| MHC-divergence^2 | 0.003 | 0.014 | -0.024 | 0.030 |
| MHC-divergence^2 : B-chick[a] | 0.010 | 0.027 | -0.044 | 0.064 |

[a] Relative to first-hatched A-chicks
[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Pair | 0.237 | 0.487 |
| Year | 0.125 | 0.354 |

**Table S44:** a) the subset of 4 models including MHC-II divergence, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick mortality, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order | 38 | -750.15 | 1586.08 | 0.00 | 0.53 |
| MHC-divergence^2 + Hatching order | 39 | -750.17 | 1588.19 | 2.11 | 0.19 |
| MHC-divergence + Hatching order | 39 | -750.14 | 1588.20 | 2.12 | 0.18 |
| MHC-divergence * Hatching order | 40 | -749.64 | 1589.46 | 3.38 | 0.10 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| **B-chick[a]** | **0.913** | **0.182** | **0.556** | **1.270** |
| MHC-divergence^2 | -0.002 | 0.028 | -0.056 | 0.053 |
| MHC-divergence | 0.027 | 0.118 | -0.204 | 0.258 |
| MHC-divergence : B-chick[a] | -0.165 | 0.182 | -0.522 | 0.192 |

[a] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Clutch | 0.001 | 0.020 |
| Pair | 0.290 | 0.538 |
| Year | 1.701 | 1.304 |

**Table S45:** a) the subset of 5 models including the number of MHC alleles, with ΔAICc < 4 relative to the best model among the 20 models considered to explain the age of first infection by ticks during the nestling stage in chicks, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. The clutch ID random effect was removed from models because associated variance estimates were virtually zero. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Null model | 18 | -464.23 | 972.47 | 0.00 | 0.45 |
| MHC-divergence^2 | 19 | -464.37 | 974.40 | 1.93 | 0.17 |
| MHC-divergence | 19 | -464.20 | 974.73 | 2.27 | 0.15 |
| Sex | 19 | -464.20 | 974.85 | 2.38 | 0.14 |
| Hatching order | 20 | -463.68 | 975.60 | 3.13 | 0.09 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| B-chick[a] | 0.135 | 0.213 | -0.282 | 0.552 |
| MHC-divergence^2 | 0.006 | 0.070 | -0.131 | 0.143 |
| Male[b] | -0.010 | 0.211 | -0.424 | 0.405 |
| MHC-divergence | -0.005 | 0.114 | -0.217 | 0.228 |

[a] Relative to first-hatched A-chicks

[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Pair | 0.222 | 0.471 |
| Year | 0.122 | 0.350 |

**Table S46:** a) the subset of 6 models including the number of MHC alleles, with ΔAICc < 4 relative to the best model among the 20 models considered to explain tick loss, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. The clutch ID random effect was removed from models because associated variance estimates were virtually zero. Abbreviations are described in Table 1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Null model | 24 | -231.32 | 532.71 | 0.00 | 0.46 |
| MHC-divergence^2 | 25 | -231.51 | 534.79 | 2.08 | 0.16 |
| Sex | 25 | -231.35 | 535.67 | 2.96 | 0.10 |
| Hatching order : MHC-divergence^2 | 24 | -232.35 | 535.69 | 2.98 | 0.10 |
| Hatching order | 25 | -231.30 | 535.79 | 3.08 | 0.10 |
| MHC-divergence | 25 | -230.89 | 536.37 | 3.65 | 0.07 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| MHC-divergence^2 | -0.019 | 0.130 | -0.273 | 0.236 |
| Male[a] | 0.027 | 0.299 | -0.560 | 0.614 |
| B-chick[b] | -0.058 | 0.673 | -0.790 | 0.674 |
| MHC-divergence | 0.060 | 0.148 | -0.231 | 0.351 |
| MHC-divergence^2: B-chick[b] | 0.023 | 0.239 | -0.239 | 0.697 |

[a] Relative to females

[b] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Pair | 0.662 | 0.814 |
| Year | 0.144 | 0.379 |

**Table S47:** a) the subset of 4 models including the number of MHC alleles, with ΔAICc < 4 relative to the best model among the 11 models considered to explain the maximum number of ticks carried by chicks during the nestling stage, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. The clutch ID random effect was removed from models because associated variance estimates were virtually zero. We used zero-truncated models with a Poisson distribution. Abbreviations are described in Table 1.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Null model | 3 | -208.16 | 422.56 | 0.00 | 0.37 |
| MHC-divergence^2 | 4 | -208.02 | 424.41 | 1.86 | 0.14 |
| Hatching order | 4 | -208.07 | 424.53 | 1.98 | 0.14 |
| Sex | 4 | -208.15 | 424.67 | 2.12 | 0.13 |
| MHC-divergence | 4 | -208.16 | 424.70 | 2.14 | 0.12 |
| Hatching order + MHC-divergence^2 | 5 | -207.92 | 426.41 | 3.85 | 0.05 |
| Sex + MHC-divergence^2 | 5 | -207.98 | 426.53 | 3.98 | 0.05 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | 0.650 | 0.326 | 0.004 | 1.295 |
| MHC-divergence^2 | -0.051 | 0.094 | -0.237 | 0.134 |
| B-chick[a] | -0.081 | 0.188 | -0.453 | 0.291 |
| Male[b] | -0.046 | 0.213 | -0.467 | 0.375 |
| MHC-divergence | -0.012 | 0.116 | -0.241 | 0.217 |

[a] Relative to first-hatched A-chicks
[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Pair | 1.266 | 1.125 |
| Year | 0.200 | 0.448 |

**Table S48:** a) the subset of 6 models including MHC-II divergence, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body size growth rate over the first 10 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. The clutch ID random effect was removed from models because associated variance estimates were virtually zero. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order | 5 | -367.23 | 744.66 | 0.00 | 0.44 |
| Hatching order + MHC-divergence | 6 | -367.20 | 746.70 | 2.03 | 0.16 |
| Hatching order+ MHC-divergence^2 | 6 | -367.21 | 746.72 | 2.05 | 0.16 |
| Hatching order + MHC-divergence + Sex | 7 | -366.76 | 747.92 | 3.26 | 0.09 |
| Hatching order + MHC-divergence^2 + Sex | 7 | -366.78 | 747.95 | 3.29 | 0.08 |
| Hatching order * MHC-divergence^2 | 7 | -366.85 | 748.10 | 3.43 | 0.08 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.273 | 0.239 | -0.743 | 0.197 |
| **B-chick[a]** | **-0.278** | **0.093** | **-0.461** | **-0.096** |
| MHC-divergence | 0.011 | 0.050 | -0.087 | 0.110 |
| MHC-divergence^2 | 0.005 | 0.020 | -0.035 | 0.045 |
| Male[b] | 0.087 | 0.093 | -0.095 | 0.270 |
| MHC-divergence^2 : B-chick[a] | -0.048 | 0.056 | -0.158 | 0.063 |

[a] Relative to first-hatched A-chicks
[b] Relative to females

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Pair | 0.279 | 0.528 |
| Year | 0.445 | 0.667 |

**Table S49:** a) the subset of 7 models including MHC-II divergence, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body mass growth rate over 35 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. The clutch ID random effect was removed from models because associated variance estimates were virtually zero. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order + MHC-divergence^2 + Sex | 7 | -277.26 | 569.07 | 0.00 | 0.25 |
| Sex | 5 | -279.39 | 569.08 | 0.01 | 0.25 |
| Hatching order+ MHC-divergence + Sex | 7 | -277.52 | 569.59 | 0.52 | 0.20 |
| MHC-divergence^2 + Sex | 6 | -279.09 | 570.60 | 1.52 | 0.12 |
| MHC-divergence + Sex | 6 | -279.33 | 571.09 | 2.01 | 0.09 |
| MHC-divergence^2 * Sex | 7 | -278.96 | 572.48 | 3.41 | 0.05 |
| MHC-divergence * Sex | 7 | -279.13 | 572.81 | 3.74 | 0.04 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.417 | 0.199 | -0.809 | -0.024 |
| MHC-divergence^2 | -0.022 | 0.037 | -0.096 | 0.051 |
| **Male[a]** | **0.488** | **0.125** | **0.243** | **0.734** |
| **B-chick[b]** | **-0.247** | **0.124** | **-0.491** | **-0.003** |
| MHC-divergence | -0.030 | 0.072 | -0.172 | 0.113 |
| MHC-divergence^2 : Male[a] | 0.045 | 0.087 | -0.128 | 0.218 |
| MHC-divergence : Male[a] | 0.087 | 0.133 | -0.175 | 0.349 |

[a] Relative to females

[b] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Pair | 0.165 | 0.406 |
| Year | 0.235 | 0.485 |

**Table S50:** a) the subset of 2 models including MHC-II divergence, with ΔAICc < 4 relative to the best model among the 20 models considered to explain chick body size growth rate over 35 days, b) model-averaged estimates for all parameters in this subset of models and c) variance and standard deviation associated with random effects in the best model. The clutch ID random effect was removed from models because associated variance estimates were virtually zero. Abbreviations are described in Table 1. Estimates different from zero are in bold.

**a)**

| Model | df | logLik | AICc | ΔAICc | ωAICc |
|---|---|---|---|---|---|
| Hatching order + MHC-divergence + Sex | 7 | -254.15 | 522.86 | 0.00 | 0.61 |
| Hatching order + MHC-divergence^2 + Sex | 7 | -254.60 | 523.76 | 0.90 | 0.39 |

**b)**

| Parameter | Std. estimate | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| Intercept | -0.319 | 0.249 | -0.811 | 0.173 |
| MHC-divergence | 0.054 | 0.056 | -0.057 | 0.165 |
| Male[a] | 0.392 | 0.110 | 0.175 | 0.610 |
| **B-chick[b]** | **-0.341** | **0.113** | **-0.564** | **-0.118** |
| MHC-divergence^2 | 0.003 | 0.020 | -0.036 | 0.042 |

[a] Relative to females

[b] Relative to first-hatched A-chicks

**c)**

| Random effect | Variance | SD |
|---|---|---|
| Pair | 0.094 | 0.306 |
| Year | 0.432 | 0.657 |

# CHAPTER 2: MHC-II distance between parents drives sex allocation decisions in a genetically monogamous wild bird

Manuscript in preparation

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**ABSTRACT**

Theory predicts that parental heritable characteristics should shape sex allocation decisions when their effects on reproduction or survival are offspring sex-dependent. Numerous studies have questioned to what extent characteristics displayed by one of the parents matched theoretical expectations. This contrasts with the handful of studies that investigated whether compatibility between parents could also trigger selective pressures for sex allocation adjustments. We studied the genetically monogamous black-legged kittiwake (*Rissa tridactyla*), where previous data revealed that female chicks suffered higher fitness costs from low diversity at genes of the major histocompatibility complex (MHC) than male chicks. We predicted, and found in our dataset, that MHC-similar parents, producing low MHC-diverse offspring, should avoid the production of females. This suggests that the genetically monogamous black-legged kittiwake parents circumvent the costs of suboptimal pairing by manipulating offspring sex. *Keywords:* compatibility; heterozygote advantage; MHC; monogamy; sex allocation

**INTRODUCTION**

Sex allocation theory predicts that parents should adjust their investment in daughters and sons depending on the fitness costs and benefits associated with each sex (Charnov, 1982; Frank, 1990; Trivers & Willard, 1973). Published data and theoretical models revealed that such sex-specific costs-benefits ratios are shaped by diverse abiotic and biotic parameters (reviewed in West, 2009). These include parental heritable genetic or non-genetic characteristics when their effects on reproduction or survival are offspring sex-dependent (Cockburn, Legge, & Double, 2002; West, 2009, chapter 6). One textbook example refers to situations where sons inherit secondary sexual characters from their father (e.g. Burley, 1981). Hence, in species where

males' elaborate ornaments translate into increased reproductive success, models predict (e.g. Pen & Weissing, 2000) and data overall suggest (Booksmythe et al., 2017; West, 2009, chapter 6) that pairs including a sexy male overproduce sons.

Besides individual parental characteristics, only a handful of studies investigated whether compatibility between parents could also trigger selective pressures for sex allocation adjustments (Brekke et al., 2010; Pryke & Griffith, 2009a, 2009b; Rioux-Paquette et al., 2011; Sardell & DuVal, 2014). This possibility was elegantly highlighted in Gouldian finches (*Erythrura gouldiae*), where daughters suffer higher viability costs from a Z-linked genetic incompatibility between red and black color morphs than sons (Pryke & Griffith, 2009a, 2009b). As predicted by sex allocation theory, females paired with a genetically incompatible male (i.e. an opposite-color morph) overproduced sons (Pryke & Griffith, 2009a).

The major histocompatibility complex (hereafter, MHC) is a key group of genes involved in the activation of immune responses against parasites (Murphy & Weaver, 2017). Here also, compatibility between parents plays a pivotal role in an evolutionary context as MHC-dissimilar mates are more likely to produce offspring carrying a higher diversity of MHC-alleles (Setchell et al., 2013), thereby able to recognize and eliminate a broader range of pathogens (Doherty & Zinkernagel, 1975; Oliver et al., 2009; Wakeland et al., 1990). This increased resistance to diseases ultimately translates into an overall higher reproductive success and survival for more MHC-diverse individuals (Brouwer et al., 2010; Lenz et al., 2013; Thoss et al., 2011; Wedekind, 1994). Some previous results revealed that sex could modulate the association between MHC-diversity and fitness, with males (Roved et al., 2018; Schaschl et al., 2012) or females (Hoover et al., 2018; this thesis, chapter 1) suffering increased fitness costs from low MHC-diversity compared to the other sex. In a sex allocation context, this predicts that parents able to adjust offspring sex in relation to the expected fitness return of either sex

given their MHC-compatibility should be advantaged. Although the MHC has been a trending topic in evolutionary ecology for two decades (Kamiya et al., 2014; Milinski, 2006), no study has yet investigated whether MHC-compatibility between parents could drive sex allocation decisions.

We investigated MHC-based sex allocation decision in the genetically monogamous black-legged kittiwake (*Rissa tridactyla*), a species in which MHC-II diversity is positively associated with growth and tick loss in female chicks, but not male chicks (this thesis, chapter 1). Both growth rate (Vincenzi et al., 2015) and tick infection (Chastel et al., 1987; McCoy et al., 2002) are known to strongly affect fitness in this species. Additionally, MHC-II diversity is positively associated with survival in second-hatched female chicks (two eggs being the typical clutch size in kittiwakes) while it is not associated with survival in any other sex-rank chick categories (this thesis, chapter 1). We therefore predicted that by producing low MHC-II diverse chicks, MHC-II similar parents should overproduce sons, especially at the second position of the laying sequence.

## MATERIALS AND METHODS

### *Study site*

The study was conducted during the 2009-2013 and 2016-2018 breeding seasons (May-August) on a colony of black-legged kittiwakes nesting on an abandoned U.S. Air Force radar tower on Middleton Island (59°26'N, 146°20'W), Gulf of Alaska. The nest sites created on the upper walls of the tower can be observed from inside through sliding one-way mirrors and birds can be individually identified using color and metal bands (Gill & Hatch, 2002). Nest sites were checked twice daily (9:00 and 18:00) to record laying and hatching events. On the day of laying,

A- and B-eggs (first-and second-laid eggs, respectively) were labeled individually with a non-toxic marker.

*Sexing procedure and sample size*

Analyses focused on two-eggs clutches (range 1-3), which is by far the most common clutch size in this population (Gill & Hatch, 2002; 78% of the clutches in these study years), and on clutches whose two parents were genotyped for MHC-II. In total, we used data from 293 pairs that produced 548 two-eggs clutches, totaling 933 chicks and 163 unhatched eggs. We sexed 913 out of these 933 chicks (97% of chicks) using DNA extracted from a drop of blood collected from the metatarsal vein a few hours after hatching (see Merkling et al., 2012 for a detailed protocol) and sexed 45 extra embryos out of the 163 unhatched eggs (27% of unhatched eggs) using DNA extracted from tissues or blood vessels from eggshells (Merkling et al., 2012). These 958 sexed embryos or chicks were used in our main analysis relating chick sex to MHC-II distance between parents. As detailed below, our statistical analyses were performed using either complete or incomplete broods, following recommendations (Krackow & Neuhauser, 2008). Finally, the relationship between MHC-II distance between parents and chick MHC-II diversity was investigated using a subsample of those chicks (n = 471) that had been sequenced for the MHC-II as part of our previous study (this thesis, chapter 1), using the protocol described next paragraph.

*Molecular analysis of MHC-II*

The DNA samples were used to amplify 258 bp fragments (218 bp excluding primers) of the exon 2 of the black-legged kittiwake MHC class-IIB. Samples were sequenced in two runs with an Illumina MiSeq platform, using the 2 × 300 bp protocol (Fasteris SA, Plan-les-Ouates, Switzerland; see chapter 1 for a detailed sequencing protocol). The reproducibility of genotype

between the two runs (n = 25 DNA samples that were split and processed in independent PCRs) was 100%. We obtained 83 different MHC class II alleles and, in the subsample used in this study, the mean number of alleles per individual was 3.29 ± 0.76 (± sd; range: 1-5).

We calculated the functional MHC-II distance between mates in pairs for which the MHC class-IIB region was sequenced for both mates, using the approach described in Strandh et al. (2012). To obtain functional alleles, we translated MHC-II DNA sequences into amino acid sequences and considered DNA sequences as functionally identical if they had the same amino-acids in the peptide-binding regions (PBRs; inferred from Leclaire et al., 2014). This gives us a total of 68 functional alleles. To calculate functional distance, we first follow the approach of Schwensow et al. (2007) to describe the chemical binding properties of each amino acid in the PBRs using five physico-chemical descriptors (z-descriptors; Sandberg et al., 1998). Then, following the approach of Strandh et al. (2012), the resulting Sandberg matrix was used to construct an alternative maximum-likelihood phylogenetic tree with "Rcontml" in the R package *Rphylip* (Revell & Chamberlain, 2014). This tree represents clusters of functionally-similar MHC sequences and was used as a reference to calculate the functional distance between MHC-sequence repertoires of parents with unweighted UniFrac analyses ("GUniFrac" package in R; Chen, 2018). Functional MHC-II distance between parents varied from 0 to 1 (mean ± sd: 0.54 ± 0.19) and did not significantly vary among years (Kruskal-Wallis, $U = 7.15$, $df = 7$, $p = 0.41$). The tree was also used to calculate the functional diversity of sexed offspring we sequenced for the MHC-II in a previous study (n = 471; this thesis, chapter 1). To calculate functional MHC-II diversity, we used the minimum total length of all the branches required to span an offspring's MHC-II alleles (i.e., Faith's phylogenetic diversity; Faith, 1992) with the R function "pd" in the *picante* R package (Kembel et al., 2010). In other words, for each additional allele, only the part of the peptide-binding characteristics that is not shared with other alleles is

summed (this thesis, chapter 1). Offspring functional MHC-II diversity varied from 0.89 to 9.81 (mean ± sd: 5.97 ± 1.12) and did not significantly vary among years (Kruskal-Wallis, U = 2.70, df = 6, p = 0.85).

*Statistical analyses*

First, we tested whether MHC-II diversity of offspring was positively associated with MHC-II distance between parents using linear mixed models (LMMs) built in the *lme4* package (Bates et al., 2015) in R 4.0.1 (R Core Team, 2020). Predictor variables included MHC-II distance between parents, offspring sex, egg rank and interactions between these variables, as well as year as a continuous variable. Clutch ID and pair ID were included as random effects to consider the non-independence of chicks born during the same breeding season or born from the same parents in the same or different years. However, variance estimates of the clutch ID random effect was practically zero and was thus removed. We standardized fixed variables by centering and dividing them by two standard deviations using the *arm* package (Gelman & Su, 2018). Model selection followed a backward-stepwise approach using the "step" function with Kenward-Roger's approximation of denominator degrees of freedom in the R package *lmerTest* (Kuznetsova, Brockhoff, & Christensen, 2017). We checked for normality and homoscedasticity of residuals and for normal distribution of random effects in the initial model.

Then, we used the same backward-stepwise approach to test the association between offspring sex and MHC-II distance between parents and whether it depended on egg rank, as predicted. We performed the same analyses on two datasets, an "unrestricted dataset" (N = 958) containing both complete (where both offspring had been sexed) and incomplete clutches (where only one offspring had been sexed), and a "restricted dataset" (N = 820) containing only complete broods. We also re-ran analyses twice on a modified form of our unrestricted dataset

by assuming that all unsexed offspring (N = 138) were females or, alternatively, males. These additional analyses allowed us to investigate whether the reported patterns could result from sex bias in mortality and/or sexing success. We built generalized linear mixed models (GLMMs) with a binomial error distribution and a logit link function (i.e. offspring sex was either 0 = female or 1 = male) in the *lme4* package (Bates et al., 2015). Predictor variables included MHC-II distance between parents, the square of that MHC-II distance, egg rank and two-way interactions between each MHC variable and egg rank, as well as year as a continuous variable, given that we previously found an increase in the probability of producing sons with time (Merkling, Hatch, Leclaire, Danchin, & Blanchard, 2019). We also included Clutch ID and pair ID as random effects and we checked for normal distribution of these random effects in the initial model. We standardized fixed variables using the *arm* package (Gelman & Su, 2018). We included a quadratic effect of parental MHC-II distance in the models because the sex-specific association between MHC-II diversity and fitness in kittiwake chicks was mainly explained by low MHC-II diverse females suffering greater fitness costs than low MHC-II diverse males, while no such sex differences was detected in highly MHC-II diverse offspring (this thesis, chapter 1). Therefore, we expected a male-biased sex ratio in pairs producing low MHC-II diverse offspring (i.e. MHC-II similar pairs) and a balanced sex ratio in pairs producing high MHC-II diverse offspring (i.e. MHC-II dissimilar pairs). We thus expected the association between offspring sex and parental MHC-II distance to be quadratic. We assessed significance of each predictor variable by the change in deviance after removal of that variable (Likelihood-Ratio Test, LRT) using a chi-square test. A variable was eliminated from the model if $p > 0.05$. Since the quadratic effect of parental MHC-II distance best explained chick sex (see results), we wanted to identify the level(s) of MHC-II distance at which the slope changed (i.e. breakpoint) and whether offspring sex ratio significantly changed on either side of the

breakpoint. We thus ran a piecewise regression model using the *segmented* R package (Muggeo, 2008) to identify the breakpoint and performed a pseudo score test to confirm that the two slopes differed significantly. Random effects cannot be considered in such piecewise analysis. Including them would probably not have changed the results as they did not have a significant effect on chick sex based on LRTs in GLMMs ($p > 0.45$).

## RESULTS

Offspring MHC-II diversity was positively associated with the MHC-II distance between parents. All other explicative variables were lost in the backward-stepwise procedure (Figure 1; Table 1). This analysis may face collinearity issues since chick sex was related to MHC-II distance between parents in our data (see the test of our main prediction below), both parameters being included concomitantly into the initial model. However, variance inflated factor (VIF) values were < 2, indicating no collinearity issue (Zuur, Ieno, & Elphick, 2010).

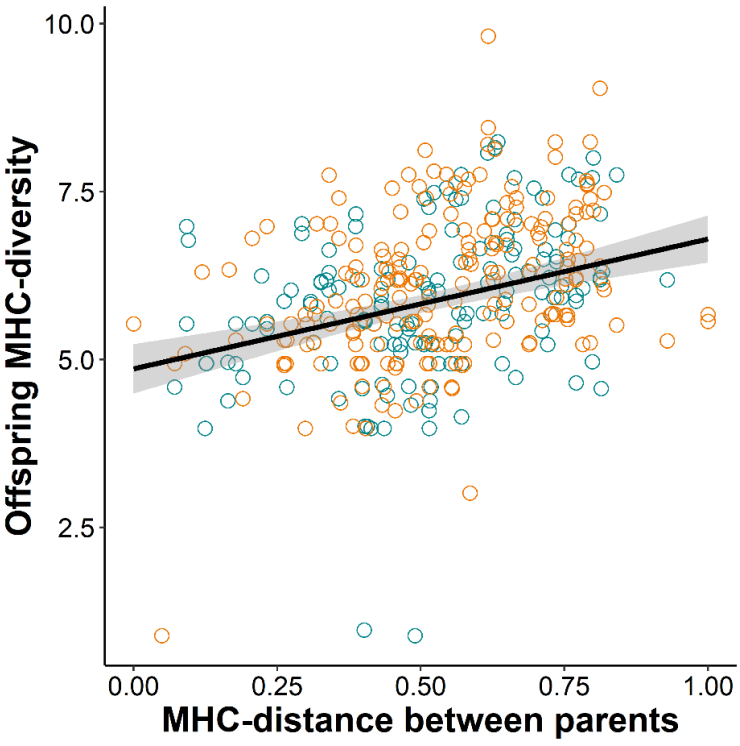


**Figure 1.** Offspring MHC-II diversity covaries with the MHC-II distance between parents in both female (orange) and male (blue) offspring. The line shows the predictions from a LMM including MHC-II distance between parents as a predictor variable. There was no significant interaction between offspring sex and MHC-II distance. The pair ID random effect was not considered in the models used for graphic representations but was accounted for in the analysis. Removing the three extremely low MHC-II diverse offspring did not change the results. Shaded areas represent confidence intervals.

**Table 1:** a) Effect of predictor variables from the generalized linear mixed model built to explain chick MHC-II diversity and b) variance and standard deviation associated with random effects in the final model. Variables were eliminated following a backward-stepwise procedure. Step denotes the exclusion sequence of the non-significant terms of the model. Values for excluded variables refer to the step before their exclusion. Values included in the final model are in bold.

**a)**

| Parameter | Estimate | SE | F | P | Step |
|---|---|---|---|---|---|
| Year | -0.048 | 0.101 | 0.225 | 0.636 | 1 |
| MHC-II distance : Sex : Hatching order | 0.027 | 0.336 | 0.625 | 0.430 | 2 |
| MHC-II distance : Hatching order | 0.028 | 0.144 | 0.038 | 0.844 | 3 |
| Sex : Hatching order | 0.043 | 0.163 | 0.069 | 0.792 | 4 |
| Hatching order | 0.014 | 0.072 | 0.035 | 0.851 | 5 |
| MHC-II distance : Sex | 0.070 | 0.162 | 0.188 | 0.665 | 6 |
| Sex | -0.121 | 0.081 | 2.216 | 0.137 | 7 |
| **MHC-II distance** | **0.859** | **0.134** | **41.230** | **< 0.001** | |

**b)**

| Random effect | Variance | SD |
|---|---|---|
| Pair ID | 0.631 | 0.794 |

We then investigated our main prediction. Using the unrestricted dataset (containing both complete and incomplete clutches), offspring sex was significantly associated with the square of MHC-II distance while the other predictor variables were eliminated in the backward-stepwise procedure (Table 2). As expected, more MHC-II similar pairs overproduced sons (Figure 2). Among offspring produced by the most MHC-II similar pairs (i.e. first of 30-quantiles), 25/37 (68%) were sons. The piecewise regression analysis identified one breakpoint (Pscore = 2.44, p = 0.01), indicating that production of sons significantly decreased until an MHC-II distance of 0.64 ± 0.06 SE (β = -1.23 ± 0.57 SE, 95% CI: -2.35, -0.12), and tended to

increase in more MHC-II dissimilar pairs, although it was not significant (β = 2.87 ± 1.77 SE, 95% CI: -0.59, 6.34; Figure S1).

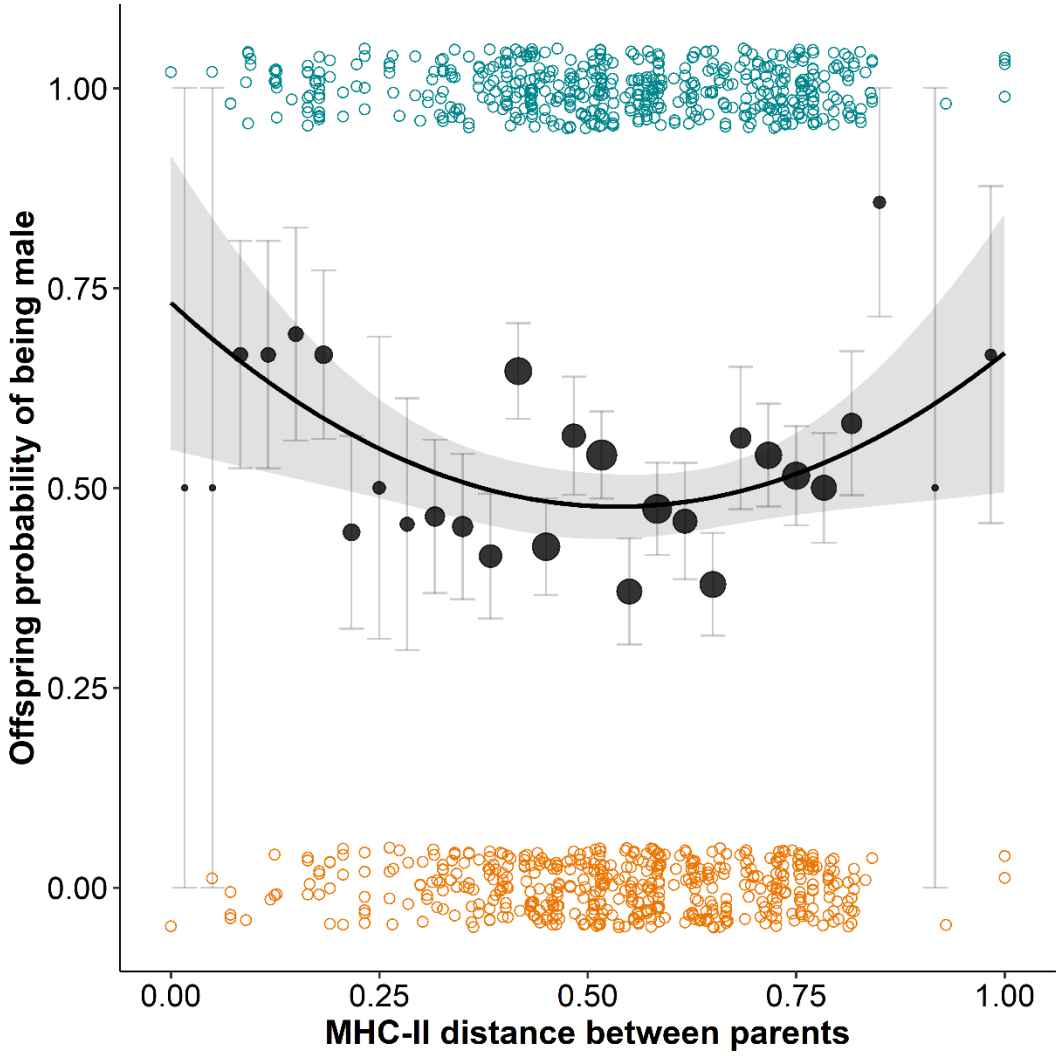


**Figure 2.** Offspring probability of being male according to MHC-II distance between parents. Each colored dot represents a female chick (orange; n = 472) or a male chick (blue; n = 486). For illustrative purpose, parental MHC-II distance was divided into 30 categories of equal range (0.033), with the black dots representing the mean (± SE) sex ratio per category of parental MHC-II distance, and the size of the dots representing sample size per category. The curve represents predicted values derived from a model including the square of MHC-II distance. Shaded areas represent 95% confidence intervals. Random effects (pair ID and clutch ID) were not considered in this model used for graphic representation. Note: the vertical position of colored dots was randomly rearranged to better appreciate the number of chicks in relation to parental MHC-II distance.

**Table 2:** a) Effect of predictor variables from the generalized linear mixed model built to explain chick sex and b) variance and standard deviation associated with random effects in the final model. Variables were eliminated following a backward-stepwise procedure. Step denotes the exclusion sequence of the non-significant terms of the model. Values for excluded variables refer to the step before their exclusion. Values included in the final model are in bold.

**a)**

| Parameter | Estimate | SE | $Chi^2$ | P | Step |
|---|---|---|---|---|---|
| MHC-II distance : Hatching order | -0.018 | 0.279 | 0.004 | 0.949 | 1 |
| MHC-II distance | 0.008 | 0.147 | 0.003 | 0.956 | 2 |
| MHC-II distance^2 : Hatching order | 0.313 | 0.415 | 0.574 | 0.449 | 3 |
| Year | -0.137 | 0.136 | 1.012 | 0.314 | 4 |
| Hatching order | 0.220 | 0.132 | 2.786 | 0.095 | 5 |
| **MHC-II distance^2** | **0.519** | **0.214** | **6.080** | **0.014** | |

**b)**

| Random effect | Variance | SD |
|---|---|---|
| Pair ID | 0.082 | 0.287 |
| Clutch ID | 0.024 | 0.156 |

The analyses performed on the restricted dataset (containing only complete clutches) gave similar results (Table S1). Furthermore, assuming that all unsexed offspring were females, or alternatively males, both lead to the same conclusion of an overproduction of males in more MHC-II similar pairs (Tables S2, S3).

## DISCUSSION

Our data first confirmed that MHC-II similar kittiwake parents were more likely to produce offspring with low MHC-II diversity. Previous results (this thesis, chapter 1) reported that such a low MHC-II diversity in offspring was associated with slower growth and reduced tick resistance in daughters only, and with increased mortality in daughters hatched in second

position as compared to other chick sex-rank categories. In line with sex allocation theory (Cockburn et al., 2002; West, 2009, chapter 6), our data revealed that in such a context, MHC-II similar parents avoided production of disadvantaged daughters. Contrary to our expectation, however, we did not find hatching rank to further modulate the association between parental MHC-II distance and offspring sex. The overall increased detrimental effect of low MHC-II diversity in daughters as compared to sons (this thesis, chapter 1) may have concealed more subtle patterns. Unexpectedly, our data also revealed a non-significant trend for MHC-dissimilar pairs to overproduce sons. This may lead to an increased fitness return if MHC-II diverse males have increased survival or reproductive advantages compared to MHC-II diverse females later in life, as shown in other species (Roved et al., 2018; Sauermann et al., 2001; Schaschl et al., 2012).

Sex allocation based on MHC similarity between parents has been suggested in humans, rats and mice because newborn males have been found to be more MHC-diverse than newborn females in these species (Dorak et al., 2002, and references therein). However, whether this result was caused by MHC-similar parents overproducing daughters has not yet been investigated. The potential adaptive value of this pattern (e.g. whether males suffered more from low MHC-diversity than females) also remains overlooked (Roved et al., 2018; Sauermann et al., 2001; Schaschl et al., 2012). Clearly, important next steps should involve studies investigating potential fitness pathways and proximate mechanisms underlying sex ratio departure from parity.

Proximate mechanisms of sex ratio adjustments are not well understood and how these could depend on MHC is unknown. Regardless of the parent(s) biasing offspring sex, our results may suggest that kittiwakes can assess the genetic characteristics of their mate (as suggested by Mulard et al., 2009; this thesis, chapter 3). The covariation between scent-gland compounds

and MHC in this species may suggest that odor cues might be used in MHC recognition (Leclaire et al., 2014), as found in several taxa (Olsson et al., 2003; Radwan et al., 2008; Wedekind et al., 1995), including birds (Leclaire et al., 2017). Sex ratio adjustments may also be the result of MHC-specific sperm-ova interactions (Wedekind, 1994), in line with previous studies reporting non-random production of blastocysts according to the MHC-distance between gametes (Lenz et al., 2018; Zhu et al., 2019).

Because permanent or temporary constraints may force individuals to mate with suboptimal partners (Stutchbury & Morton, 1995; Tinghitella, Weigel, Head, & Boughman, 2013), tactics allowing to lessen associated costs may have emerged. Such constraints are particularly likely to happen in genetically monogamous species such as the kittiwake (Helfenstein, Tirard, et al., 2004). We previously reported that breeding kittiwakes flexibly adapted their breeding timing and copulatory behavior in response to within-pair genetic similarity (this thesis, chapter 3). The present study suggests another way for kittiwake parents to circumvent fitness costs associated to increased genetic similarity.

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SexCoMonArc") to S.L. and E.D. This work originated in the laboratory "Evolution et Diversité Biologique" (EDB) and was supported by the French Laboratory of Excellence Project "TULIP" (ANR-10-LABX-41; ANR-11-IDEX-0002-02), and the Agence Nationale de la Recherche Francaise (ANR grant "BactOdo", no. ANR-13-PDOC-0002 to S.L.). M.P. was supported by a French doctoral scholarship. The authors declare no conflict of interest.

**SUPPLEMENTARY MATERIAL**

*Figures*

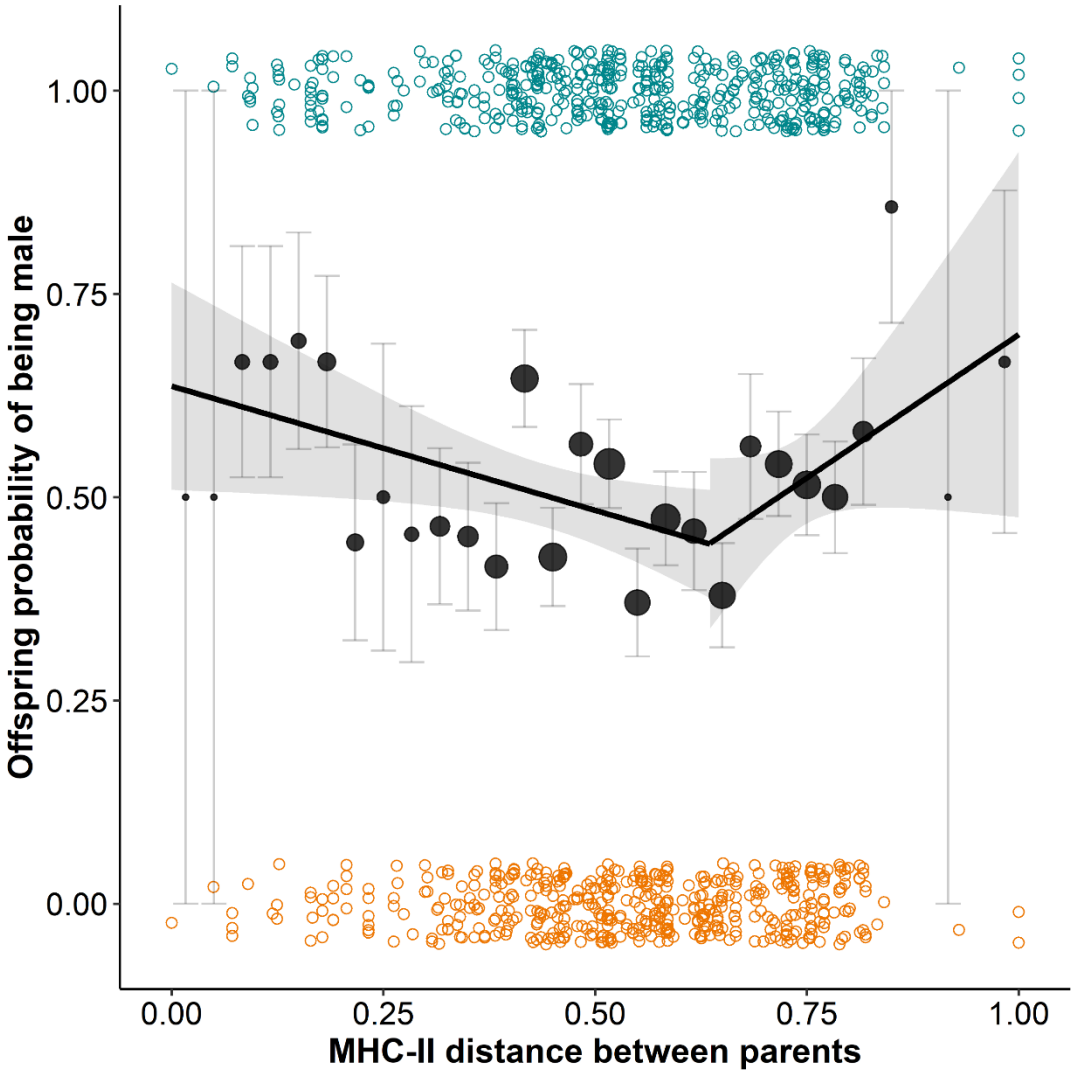


**Figure S1.** Offspring probability of being male according to MHC-II distance between parents. Each colored dot represents a female chick (orange; n = 472) or a male chick (blue; n = 486). For illustrative purpose, parental MHC-II distance was divided into 30 categories of equal range (0.033), with the black dots representing the mean (± SE) sex ratio per category of parental MHC-II distance, and the size of the dots representing sample size per category. The two solid black lines correspond to the segmented linear regressions on either side of the breakpoint obtained from the piecewise regression analysis. Shaded areas represent 95% confidence intervals. Random effects (pair ID and clutch ID) were not considered in this model used for graphic representation. Note: the vertical position of colored dots was randomly rearranged to better appreciate the number of chicks in relation to parental MHC-II distance.

*Tables*

**Table S1:** a) Effect of predictor variables from the generalized linear mixed model built to explain chick sex using the "restricted" dataset (i.e. considering complete clutches only) and b) variance and standard deviation associated with random effects in the final model. Variables were eliminated following a backward-stepwise procedure. Step denotes the exclusion sequence of the non-significant terms of the model. Values for excluded variables refer to the step before their exclusion. Values included in the final model are in bold.

**a)**

| Parameter | Estimate | SE | $Chi^2$ | P | Step |
|---|---|---|---|---|---|
| MHC-II distance : Hatching order | -0.052 | 0.301 | 0.030 | 0.863 | 1 |
| MHC-II distance | 0.037 | 0.160 | 0.055 | 0.814 | 2 |
| MHC-II distance^2 : Hatching order | 0.271 | 0.444 | 0.374 | 0.541 | 3 |
| Year | -0.125 | 0.148 | 0.712 | 0.399 | 4 |
| Hatching order | 0.213 | 0.143 | 2.233 | 0.135 | 5 |
| **MHC-II distance^2** | **0.509** | **0.233** | **4.904** | **0.027** | |

**b)**

| Random effect | Variance | SD |
|---|---|---|
| Pair ID | 0.104 | 0.322 |
| Clutch ID | 0.013 | 0.115 |

**Table S2:** a) Effect of predictor variables from the generalized linear mixed model built to explain chick sex using a modified form of our "unrestricted" dataset assuming that all unsexed offspring (N = 138) were females and b) variance and standard deviation associated with random effects in the final model. Variables were eliminated following a backward-stepwise procedure. Step denotes the exclusion sequence of the non-significant terms of the model. Values for excluded variables refer to the step before their exclusion. Values included in the final model are in bold.

**a)**

| Parameter | Estimate | SE | $Chi^2$ | P | Step |
|---|---|---|---|---|---|
| Year | -0.037 | 0.127 | 0.085 | 0.770 | 1 |
| MHC-II distance^2: Hatching order | 0.187 | 0.390 | 0.232 | 0.630 | 2 |
| MHC-II distance: Hatching order | 0.131 | 0.246 | 0.283 | 0.595 | 3 |
| MHC-II distance | 0.080 | 0.137 | 0.343 | 0.558 | 4 |
| Hatching order | 0.106 | 0.123 | 0.743 | 0.389 | 5 |
| **MHC-II distance^2** | **0.432** | **0.195** | **4.945** | **0.026** | |

**b)**

| Random effect | Variance | SD |
|---|---|---|
| Pair ID | 0.083 | 0.288 |
| Clutch ID | <0.001 | <0.001 |

**Table S3:** a) Effect of predictor variables from the generalized linear mixed model built to explain chick sex using a modified form of our "unrestricted" dataset assuming that all unsexed offspring (N = 138) were males and b) variance and standard deviation associated with random effects in the final model. Variables were eliminated following a backward-stepwise procedure. Step denotes the exclusion sequence of the non-significant terms of the model. Values for excluded variables refer to the step before their exclusion. Values included in the final model are in bold.

**a)**

| Parameter | Estimate | SE | $Chi^2$ | P | Step |
|---|---|---|---|---|---|
| MHC-II distance : Hatching order | -0.150 | 0.263 | 0.326 | 0.568 | 1 |
| MHC-II distance | -0.050 | 0.135 | 0.135 | 0.713 | 2 |
| MHC-II distance^2 : Hatching order | 0.300 | 0.393 | 0.588 | 0.443 | 3 |
| Year | -0.203 | 0.125 | 2.634 | 0.105 | 4 |
| **Hatching order** | **0.273** | **0.124** | **4.911** | **0.267** | |
| **MHC-II distance^2** | **0.473** | **0.201** | **5.795** | **0.016** | |

**b)**

| Random effect | Variance | SD |
|---|---|---|
| Pair ID | 0.483 | 0.220 |
| Clutch ID | 0.006 | 0.077 |

# CHAPTER 3: Behavioral avoidance of sperm aging depends on genetic similarity of mates in a monogamous seabird

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## ABSTRACT

Inbreeding, i.e. the mating of genetically related individuals, can lead to reduced fitness and is considered to be a major selective force of mate choice. Although inbreeding avoidance has been found in numerous taxa, individuals may face constraints when pairing, thereby mating with suboptimal partners. In such circumstances, individuals that are able to avoid factors exacerbating detrimental effects of inbreeding should be favored. Using the socially and genetically monogamous black-legged kittiwake (*Rissa tridactyla*), we explored whether the detrimental effects of inbreeding are exacerbated by sperm aging (i.e. the post-meiotic senescence of sperm cells, mainly occurring within the female tracts after copulation), and whether they can be mitigated by behavioral tactics. First, by experimentally manipulating the age of the fertilizing sperm, we found that hatching failure due to sperm aging increased with higher genetic similarity between mates. We then investigated whether more genetically similar pairs exhibited mating behaviors that prevent fertilization by old sperm. The more genetically similar mates were, the less likely they were to copulate early in the reproductive season and the more females performed postcopulatory sperm ejections. By flexibly adapting their behavior in response to within-pair genetic similarity, kittiwakes may avoid exacerbation of inbreeding costs by sperm aging.

*Keywords:* Fitness, gamete, genetic relatedness, postcopulatory choice, reproductive behaviors, sperm ageing, sperm senescence

## INTRODUCTION

Studies on reproductive strategies based on the genotypes of partners have been steadily growing (Firman, Gasparini, Manier, & Pizzari, 2017; Jennions & Petrie, 2000; Kamiya et al., 2014; Kempenaers, 2007; Neff & Pitcher, 2005). In particular, evidence has accumulated for

avoidance of inbreeding, i.e. the mating of genetically related individuals (Hoffman et al., 2007; Leclaire, Nielsen, Sharp, & Clutton-Brock, 2013; Mulard et al., 2009; Pusey & Wolf, 1996), in line with studies reporting a negative effect of inbreeding on fitness (Charlesworth & Charlesworth, 1987; DeRose & Roff, 1999; Keller & Waller, 2002). Inbreeding can reduce fitness because of the expression of detrimental recessive alleles or the loss of overdominance at loci with heterozygote advantage (Charlesworth & Charlesworth, 1987; Roff, 2002). The detrimental effects of inbreeding on fitness are exacerbated under stressful conditions (Armbruster & Reed, 2005; Fox & Reed, 2011; Ihle, Hutter, & Tschirren, 2017), including adverse abiotic factors (e.g. temperature, drought), intra-specific competition (Armbruster & Reed, 2005; Fox & Reed, 2011) and high pathogen load (Bello-Bedoy & Nunez-Farfan, 2011; Coltman et al., 1999; Ilmonen et al., 2008).

Recently, sperm aging, which is receiving growing attention in evolutionary biology (Firman, Young, Rowe, Duong, & Gasparini, 2015; C. Gasparini, Daymond, & Evans, 2018; C. Gasparini, Dosselli, & Evans, 2017; C. Gasparini, Kelley, & Evans, 2014; Pizzari, Dean, Pacey, Moore, & Bonsall, 2008; Reinhardt, 2007; Vega-Trejo, Fox, Iglesias-Carrasco, Head, & Jennions, 2019; White et al., 2008), has been shown to exacerbate inbreeding (Tan, Pizzari, & Wigby, 2013). Sperm aging refers to the post-meiotic senescence of haploid sperm cells and is independent from aging of the diploid organism (Pizzari et al., 2008). In *Drosophila melanogaster*, inbreeding decreases offspring viability when females are fertilized by old sperm but not by young sperm (Tan et al., 2013). However, the mechanisms by which sperm age and inbreeding interplay to modulate embryo survival or growth remain unclear. As they age, sperm cells accumulate damage in DNA and changes in DNA methylation profiles (Aitken & Baker, 2006; Menezo, Silvestris, Dale, & Elder, 2016; Twigg, Fulton, Gomez, Irvine, & Aitken, 1998), mainly as a result of oxidative stress (Pizzari et al., 2008; Reinhardt, 2007). These alterations

of sperm DNA have deleterious effects on fertilization potential and viability of zygotes and offspring (C. Gasparini et al., 2014; Tarin, Perez-Albala, & Cano, 2000; White et al., 2008), but can be repaired by post-fertilization mechanisms (Menezo et al., 2016). However, defection in DNA repair mechanisms may be associated with the expression of detrimental recessive alleles at genes controlling such mechanisms (Okayasu et al., 2000; Perez et al., 2007). Thus, one could speculate that an interplay between inbreeding and sperm aging may decrease embryo and offspring viability.

The increased deleterious effects of sperm aging in inbred reproductive events may have created selective pressures inducing the evolution of counter strategies. In numerous species, individuals avoid inbreeding through pre-copulatory mate choice (Hoffman et al., 2007; Leclaire et al., 2013; Mulard et al., 2009), post-copulatory strategies (Bretman et al., 2009; Pizzari et al., 2004; Welke & Schneider, 2009), or both (Daniel & Rodd, 2016; C. Gasparini & Pilastro, 2011). However, individuals do not necessarily have a choice of their sexual partner or can face permanent or temporary constraints in choosing within a limited pool of potential mates. These constraints include various ecological restrictions such as limited search areas (Frankham, 1998; Pusey & Wolf, 1996), asynchrony in reproductive phenology (Lehmann & Perrin, 2003; Stutchbury & Morton, 1995) or biased sex-ratio (Kvarnemo & Simmons, 1999; Tinghitella et al., 2013). When breeders have no option but to mate with genetically similar partners, they may limit fitness costs by avoiding factors exacerbating the deleterious effects of inbreeding. Several strategies preventing fertilization by old sperm have been proposed (Reinhardt, 2007), but only a few have been empirically described. These include female preferential selection of spermatophores containing young sperm (Reinhardt & Siva-Jothy, 2005) and sperm ejection by females following copulations occurring long before the female fertile period (Wagner et al., 2004; White et al., 2008). However, whether these strategies are

preferentially used by individuals paired with genetically similar mates (i.e. facing higher probability of suffering from fertilization by old sperm) has yet to be examined.

Here, we present evidence that sperm aging exacerbates the detrimental effects of inbreeding in the black-legged kittiwake *(Rissa tridactyla*), and report behavioral tactics that might reduce such effects. Kittiwakes are strictly monogamous during a given breeding season (Helfenstein, Tirard, et al., 2004) and frequently retain the same mate over several years, although divorce can occur after breeding failure (Naves et al., 2007). Breeding failure in kittiwakes is associated with sperm aging (Wagner et al., 2004; White et al., 2008), and might be limited by females preferentially ejecting sperm following precocious copulations (i.e. sperm that would have been old by the time of fertilization) (Wagner et al., 2004). Kittiwakes also suffer reproductive costs from inbreeding and, preferentially mate with genetically dissimilar mates (Mulard et al., 2009), possibly via an odor-based mechanism (Leclaire et al., 2012). However, not all individuals pair with a genetically dissimilar mate, maybe because of constraints on mate choice (Mulard et al., 2009). Being strictly monogamous, kittiwakes cannot avoid inbreeding through post-pairing strategies (e.g. extra-pair mating, cryptic female choice), and are thus expected to have evolved strategies that limit the factors exacerbating the deleterious effects of inbreeding. If sperm aging exacerbates the detrimental effects of inbreeding in kittiwakes, we predict therefore, that the more genetically similar mates are, the more they use the behavioral strategies preventing fertilization by aged sperm (i.e. avoidance of precocious copulations, and sperm ejection after precocious copulations).

Our long-term monitoring of kittiwake populations has created the first opportunity of which we are aware to examine the potential behavioral adaptations to interactions between sperm aging and inbreeding. First, using a well-established protocol (White et al., 2008), we manipulated the age of the fertilizing sperm to investigate whether sperm aging exacerbates the

detrimental effects of inbreeding on three proxies of fitness: eggs viability and hatchlings body condition and size (Helfenstein, 2002; Helfenstein, Danchin, & Wagner, 2004). Hence, this experimental design makes it possible to highlight fitness costs not otherwise detectable in nature if counter-strategies have evolved. Then, to determine whether behavioral strategies might mitigate these costs, we used behavioral observations conducted on unmanipulated breeding pairs in another kittiwake population and tested whether the timing of copulations and sperm ejections varied with genetic similarity between mates.

## MATERIALS AND METHODS

The manipulative part of this study was conducted in the 2006, 2009 and 2010 breeding seasons (May–August) on a colony of black-legged kittiwakes nesting on an abandoned U.S. Air Force radar tower on Middleton Island (59°26'N, 146°20'W), Gulf of Alaska. Nest sites created on the upper walls of the tower can be observed from inside through sliding one-way mirrors (Gill & Hatch, 2002). All nest sites were checked twice daily (9:00 and 18:00) to record laying and hatching events.

We used a protocol developed on kittiwakes during previous breeding seasons (White et al., 2008). Briefly, after pairs (n = 27) had commenced copulating, males were fitted with an anti-insemination ring (i.e. a rubber ring placed around the cloaca and maintained with a harness) that prevents cloacal contact and insemination, and hence females from receiving fresh sperm (White et al., 2008). Males were recaptured after completion of the clutch to remove their ring. Thus, the minimum age of sperm available for fertilization corresponded to the number of days the ring was worn before laying. Rings were fitted randomly over a period of 19 days preceding egg laying. As in most wild species, the exact duration between fertilization and egg laying is unknown in kittiwakes. We assumed that fertilization occurred between one

and two days before egg laying (Bakst, Wishart, & Brillard, 1994). Because fertilization might have occurred before the ring was fitted if the male wore a ring for less than 2 days before laying, we excluded the corresponding egg from analyses (n = 2). Rings were inconspicuous, as they were covered by surrounding feathers, and they allowed normal behavior (White et al., 2008).

Because inbreeding and sperm aging are known to have strong effects on early-life stages (Hemmings et al., 2012; Spottiswoode & Moller, 2004; White et al., 2008), we used hatching success, and chick body condition (body mass adjusted for tarsus length) and size (i.e. tarsus length) at hatching as proxies of fitness. Tarsus length is a good estimator of overall body size in adults (Rising & Somers, 1989), which is a good indicator of breeding success in kittiwakes (Helfenstein, 2002; Helfenstein, Danchin, et al., 2004). On the day of laying, A- and B- eggs (i.e. the first and the second laid egg, respectively) were labeled individually with a non-toxic marker. To facilitate egg-development monitoring, eggs were removed from their nest and placed in artificial incubators, which does not affect hatching success in kittiwakes (White et al., 2008). We checked for embryonic development using egg candling, which consists in using a bright light to see through the shell. Eggs that did not exhibit early signs of embryonic development (i.e. ‘‘yolk spreading’’; n = 4 eggs) were conservatively excluded from the analyses to ensure that sperm age, and not sperm presence, was the only factor manipulated as in White et al. (2008). This led to a final sample size of 36 eggs in 24 nests. Eggs were placed back in their nest after 25 days of incubation (the incubation period of kittiwakes lasts 27 days; J. C. Coulson & White, 1958) or as soon as external pipping occurred. Within 12 h of hatching, all chicks were weighed to the nearest gram using an electronic scale and tarsus length was measured to the nearest millimeter using a caliper (n = 24 chicks).

To control for any potential effects caused by the ring itself on reproductive performance, other males were fitted with a thinner control ring that did not prevent insemination (White et al., 2008). In this control group (n = 43 eggs and 32 chicks), we found no significant association between reproductive performance and ring wear duration (see supplementary material for details). This indicates that the potential effects on reproductive performance in the experimental group are due to sperm ageing and not to the wearing of the ring *per* se, as previously found by White et al. (2008).

*Observation of mating behavior in unmanipulated pairs*

Behavioral observations were conducted in the 1999-2001 breeding seasons (May–August) on the kittiwake population nesting at Cap Sizun in Brittany, France (48°5'N, 4°36'W), where birds can be individually identified using color bands (E. Danchin, Boulinier, & Massot, 1998).

The protocol used for behavioral observations is described in Helfenstein et al. (2004; 2003). In summary, we used daily continuous observations (Altmann, 1974) to record copulations and sperm ejections in pairs nesting on a cliff (daily number of hours of observation, mean ± sd: 3.76 ± 2.06; range: 1 to 10). We observed 13 pairs in 1999, 19 pairs in 2000 and 21 pairs in 2001 (35 unique pairs in total as 14 pairs were observed during more than one year), in which both mates were banded, genotyped, and observable from a single observation point approximately 30 m away. Sperm ejection is defined as females forcefully ejecting a white fluid within 90 s of the male dismounting. These distinctive cloacal expulsions happen non-randomly after copulation and are different from defecations, which happen without noticeable muscular contraction (Helfenstein et al., 2003). We used copulations occurring within 20 days before the laying of the first egg to allow comparison with the experimental study, and because kittiwakes

rarely mate before this period (Helfenstein, Tirard, et al., 2004). Cape Sizun birds were caught on the nest using a hook system to collect blood (Helfenstein, Tirard, et al., 2004).

*Genetic analyses*

For all birds, blood was taken from the alar vein using a 1 ml syringe and a 25-gauge needle, and kept in a preservative solution. DNA was extracted from each blood sample using either a "salting out" protocol (Mulard et al., 2009) or the DNeasy Blood and Tissue Kit (Qiagen Group) following the supplier's guidelines. Birds were genotyped at 10 microsatellite loci (Leclaire et al., 2012; Mulard et al., 2009) by using the protocol described in Mulard et al. (2009) (n = 114 birds). Additional DNA samples (n = 48 birds) were analyzed subsequently by using a more recent protocol described in Leclaire et al. (2012). The correspondence between the two methods was tested by genotyping 30 individuals using the two protocols. Correspondence did not match for loci K32 and RBG20 (Leclaire et al., 2012). Genetic relatedness between mates that were not genotyped with the same protocol (n = 6 pairs out of 81 pairs) was therefore calculated without these two loci.

We used the GENEPOP Version 4.6 (Rousset, 2008) to test linkage disequilibria and deviation from Hardy-Weinberg equilibrium (Markov chain parameters: 10000 dememorization steps, 100 batches, and 5000 iterations per batch). After correcting for multiple tests, the K16 locus appeared to be out of Hardy-Weinberg equilibrium in both population ($p < 0.001$) and no locus was genetically linked to another locus ($p > 0.05$). Therefore, we excluded K16 from the genetic relatedness analyses.

Genetic similarity between mates was calculated using the identity index ($R_{ID}$) (Mathieu, Autem, Roux, & Bonhomme, 1990) in the IDENTIX software (Belkhir, Castric, & Bonhomme, 2002). This index has been validated as a good estimator of the consanguinity of

offspring in cases where identical alleles are likely to be identical by descent, something especially relevant when interested in the fitness consequences of inbred mating (Belkhir et al., 2002). In addition, this index has been used in previous studies that revealed patterns and effects of biological meaning in kittiwakes (Leclaire et al., 2012; Mulard et al., 2009). $R_{ID}$ was transformed in an estimate of genetic distance ($D_{ID}$) using the formula, $D_{ID} = 1 - R_{ID}$ that can theoretically range from 0 (corresponding to mates sharing the same microsatellite alleles) to 1 (corresponding to mates sharing not a single microsatellite allele). In our study, $D_{ID}$ ranged from 0.39 to 0.83 (mean ± sd: 0.64 ± 0.10; n = 46 pairs) for pairs from the Middleton population, and from 0.23 to 0.61 (mean ± sd: 0.43 ± 0.09; n = 35 pairs) for pairs from the Cap Sizun population.

*Statistical analyses*

*i. Experimental manipulation of sperm age*

We tested the effect of genetic similarity and sperm age on each of the three proxies of fitness (i.e. hatching success, body condition and tarsus length at hatching) using mixed models. Explanatory variables were the genetic distance between pair members, the duration of ring wear (i.e. minimum sperm age), their two-way interaction and egg rank. Tarsus length at hatching was included in the model built for body mass at hatching, which can thus be interpreted as size-adjusted body mass, or body condition (Garcia-Berthou, 2001). Year and pair identity were included as random effects. For hatching success analyses, we used a generalized linear mixed model (GLMM) and a binomial distribution, while for body mass and tarsus length analyses, we used linear mixed models (LMMs). We checked for outliers by calculating Cook's distance with the *influence.ME* package in R (Nieuwenhuis, te Grotenhuis, & Pelzer, 2012). We considered as too influential the data points with a Cook's distance that

exceeded the cut-off value 4/n, with n being the sample size (Nieuwenhuis et al., 2012). Analyses were redone when excluding the influential data, and results were similar (see Results). For LMMs, we checked for normality and homoscedasticity of residuals.

*i. Observation of mating behavior in unmanipulated pairs*

As found in previous studies (Helfenstein, Tirard, et al., 2004; White, 2008), kittiwakes were rarely observed copulating more than once a day (18 cases over 926 observations). Therefore, to test for a relationship between genetic similarity and copulation behavior in unmanipulated pairs, we calculated the daily probability of observing at least one copulation for each pair (n = 53 pairs). We built a GLMM using a binomial distribution with this binary variable as the response variable. Over the 146 copulations recorded during this period, 38 (26%) were followed by sperm ejection, and no sperm ejection was observed between 20 and 15 days before laying. Analyses on sperm ejection were thus restricted to the 15 days before laying. We tested whether sperm ejection probability was related to genetic similarity using a GLMM with a binomial distribution. For each pair (n = 45 pairs), the proportion of copulations followed by sperm ejection on a given day was used as the response variable and was weighted by the number of copulations (using the *weights* parameter in the *glmer* function from the *lme4* package (Bates et al., 2015) in R (R Core Team, 2017)). Number of days before laying, genetic distance between pair members and their two-way interaction were included as explanatory variables in models explaining copulation and sperm ejection. Pair identity nested in year, and date were included as random effects.

In all analyses, we standardized variables before analysis, and models were fitted with a maximum likelihood estimator, and normality of the random effects was checked. The significance of a term in the model was assessed by the change in deviance after removal of

that term (Likelihood-Ratio Test, LRT) using a chi-square test. The interaction was removed when not significant. All statistical analyses were performed with R 3.4.3 (R Core Team, 2017) and the *lme4* R package (Bates et al., 2015).

## RESULTS

### *Effects of sperm age and genetic distance on fitness proxies*

Hatching success was significantly related to the interaction between genetic distance and minimum sperm age (n = 36, $\chi_1^2$ = 6.20, $p$ = 0.01; Figure 1A, B). The more genetically similar mates were, the more sperm age impaired hatching success. There was no significant effect of egg rank on hatching success ($\chi_1^2$ = 0.73, $p$ = 0.39). Similar results were obtained when influential data points were excluded (n = 33; genetic distance x minimum sperm age: $\chi_1^2$ = 17.92, $p$ < 0.001; egg rank: $\chi_1^2$ = 0.42, $p$ = 0.52). Body condition at hatching was not significantly related to any of the parameters we considered (n = 24, all $p$ > 0.36) except egg rank (n = 24, $\chi_1^2$ = 6.36, $p$ = 0.01), A-chicks being in better condition than B-chicks. Redoing this analysis without influential data points did not change the results (n = 21, all $p$ > 0.08, except for egg rank: $\chi_1^2$ = 7.47, $p$ = 0.01). Tarsus length at hatching was not significantly associated with the two-way interaction between genetic distance and minimum sperm age (n = 24, $\chi_1^2$ = 2.89, $p$ = 0.09), nor with any other parameters (all $p$ > 0.74). Redoing this analysis without influential data points did not change the results (n = 20, all $p$ > 0.25).

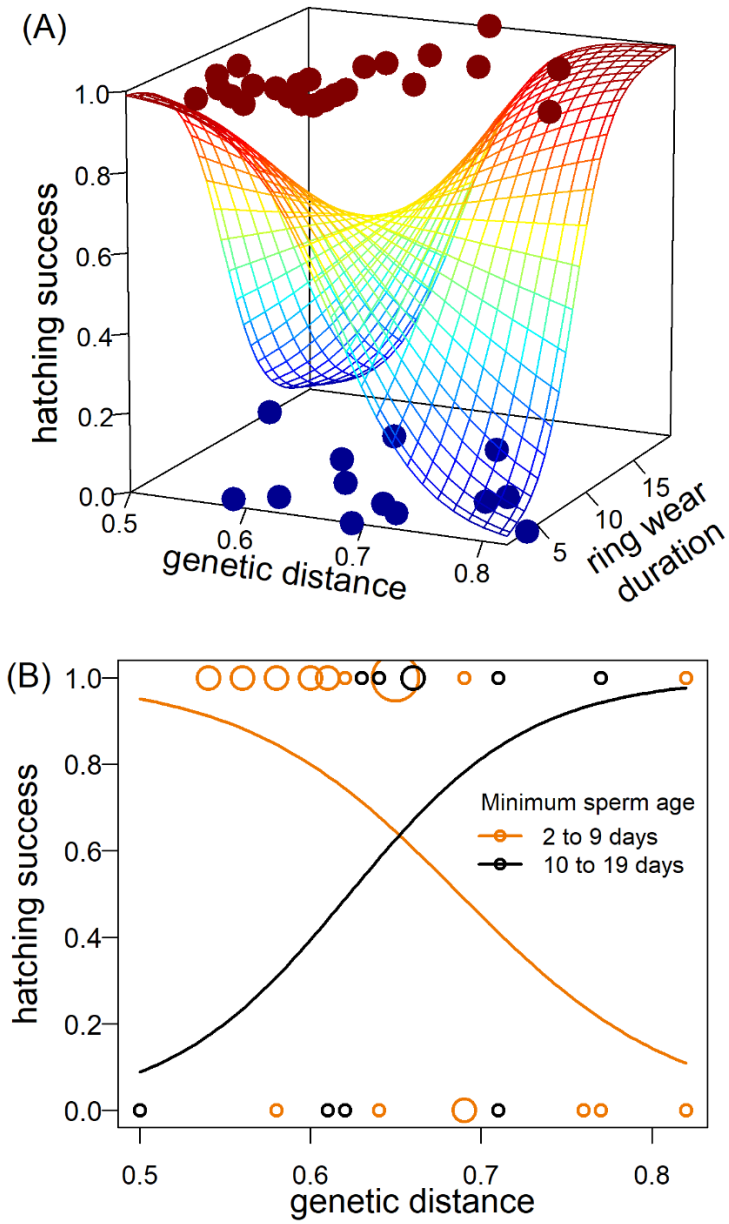


**Figure 1. Effects of genetic distance and minimum sperm age (the duration of anti-insemination ring wear) on hatching success (A) in a 3D view and (B) a 2D view.** Predicted values of hatching success were derived from the model including genetic distance between mates, the duration of ring wear and their interaction as fixed effects. For clarity, random effects (year and pair identity) were not considered in the model used for graphic representations. Removing influential data points did not change the results. In (A), each point represents whether an egg hatched (red) or not (blue) and colors of the grid represent estimated hatching success, with blue corresponding to low values, red to high values and yellow/green to intermediate values. In (B), we divided the ring wear duration (2 to 19 days) in half, producing a short ring-wear duration (2 to 9 days, orange points, n = 26 eggs) and a long ring-wear duration (10 to 19 days, black points, n = 10 eggs). The size of the points represents the number of eggs. Predicted values were derived from the model described above by fixing the duration of ring wear to 5 days (i.e. short ring wear duration, orange curve) or 15 days (i.e. long ring wear duration, black curve).

*Effects of genetic distance and time on reproductive behaviors*

The probability of copulation varied significantly with the interaction between genetic distance and the number of days before laying ($\chi_1^2$ = 7.82, $p$ = 0.005; Figure 2A, B). The probability of copulation decreased markedly with increasing pairwise genetic similarity early in the reproductive season but not as laying date approached (Figure 2A, B). The probability of sperm ejection decreased with pairwise genetic distance ($\chi_1^2$ = 4.19, p = 0.04; Figure 3A) and decreased as laying date approached ($\chi_1^2$ = 6.52, p = 0.01; Figure 3B), but it did not vary with the interaction between these two variables ($\chi_1^2$ = 0.17, p = 0.68).

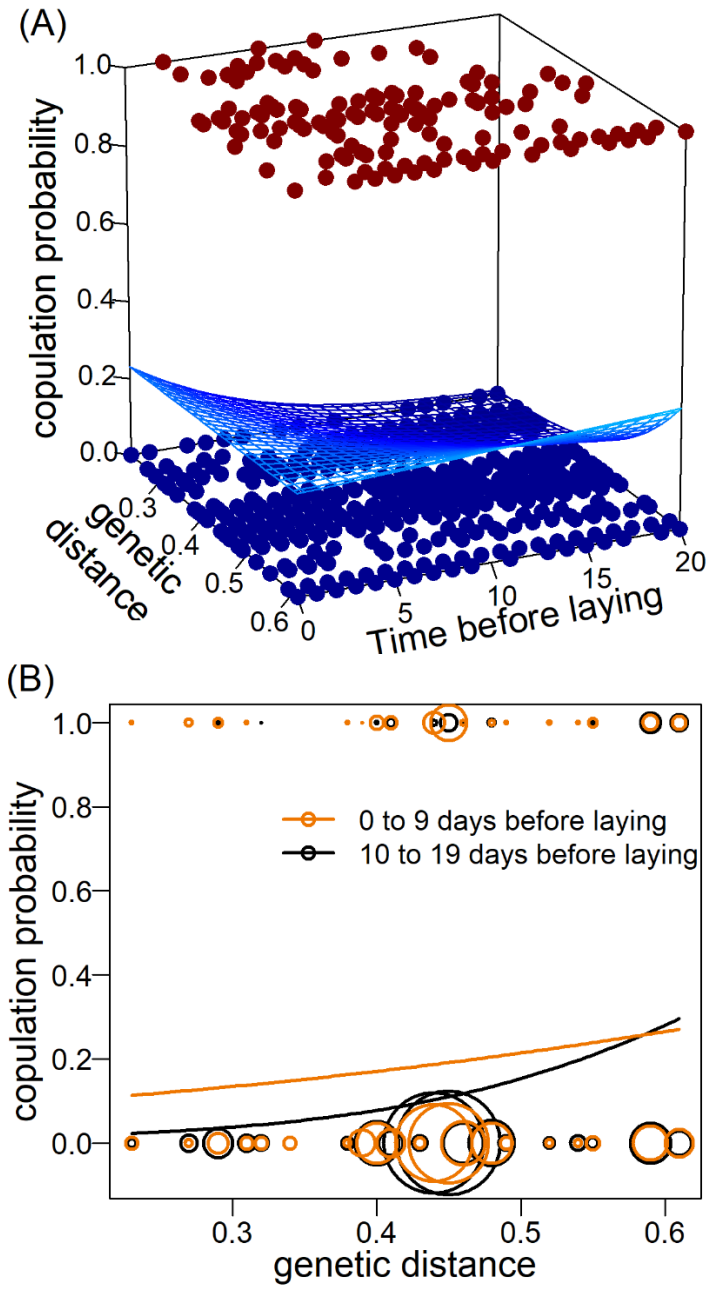


**Figure 2: The probability of copulation over time and according to genetic distance (A) in a 3D view and (B) in a 2D view.** Predicted values of copulation probability were derived from the model including genetic distance between mates, number of days before laying of the first egg and their interaction. For clarity, random effects (pair identity nested in the year and Julian day) were not considered in these models for graphic representations. In (A), each point represents whether a pair copulated (red) or not (blue) during a given day of observation. In (B), we divided the period before laying (20 to 0 days) in half, producing a late period (0 to 9 days before laying, in orange, n = 456 observations) and an early period (10 to 20 days before laying, in black, n = 470 observation). The size of the points represents the number of observations. Predicted values were calculated by fixing the time before laying to 5 days (i.e. early period, orange line) or 15 days (i.e. late period, black line).

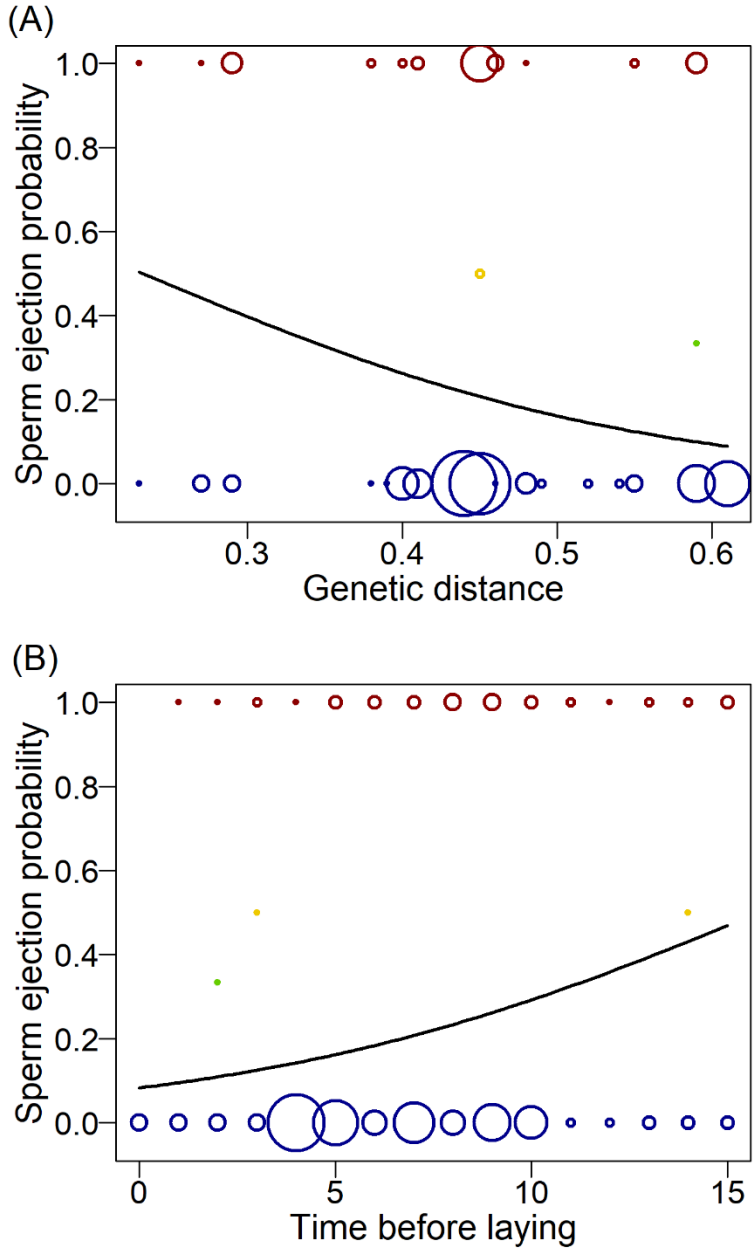


**Figure 3: Postcopulatory sperm ejection probability (A) according to genetic distance and (B) over time.** Each point represents the proportion of copulations followed by sperm ejection for a given pair on a given day and the size of the points represents the number of pairs. Kittiwakes rarely copulate more than once a day, meaning that sperm ejection probabilities were mostly equal to 1 (in red) or 0 (in blue). The regression lines represent the predicted values derived from the models including genetic distance between mates, number of days before laying of the first egg and their interaction. For clarity, random effects (pair identity nested in the year and Julian day) were not considered in these models for graphic representations. Predicted values were calculated in (A) by fixing the time before laying to the median number of days (i.e. 7 days) and in (B) by fixing genetic distance to the median value (i.e. 0.43).

## DISCUSSION

Our experimental manipulation of sperm age in kittiwakes, combined with behavioral observations of copulations and sperm ejections in unmanipulated pairs, have created a unique opportunity to investigate the deleterious interactions of sperm age and inbreeding, along with the behavioral tactics that can minimize them. We found that (i) sperm aging and genetic similarity interacted to reduce fitness in the form of decreased hatching success, and that (ii) the frequency of two behavioral strategies used to avoid fertilization by old sperm, namely avoidance of early copulations and post-copulatory sperm ejection, increased with genetic similarity between mates.

Prior studies in kittiwakes showed that hatching success was independently reduced by inbreeding (Mulard et al., 2009) and sperm aging (Wagner et al., 2004; White et al., 2008). Here, by experimentally inducing the fertilization of eggs with old sperm, we found that the decrease in hatching success associated with sperm aging increased with genetic similarity between mates, suggesting that fertilization by old sperm can exacerbate the detrimental effects of inbreeding. Our results add to those in *D. melanogaster* (Tan et al., 2013), and suggest that the deleterious interaction between sperm age and inbreeding may be found across several taxa, which may provide new insights into inbreeding-stress interactions in vertebrates (Ihle et al., 2017; Marr, Arcese, Hochachka, Reid, & Keller, 2006; Pemberton, Ellis, Pilkington, & Berenos, 2017). However, we did not detect any effects of the interplay between inbreeding and sperm aging on hatchling body condition and size. These results are in line with several studies that have reported that the effects of inbreeding and sperm aging are especially high during embryo development (Hemmings et al., 2012; Reinhardt, 2007; Spottiswoode & Moller, 2004; Tarin et al., 2000).

We observed that more genetically similar pairs copulated more frequently as egg-laying approached compared to more genetically distant pairs. In addition, females that were more genetically similar to their mate ejected sperm more frequently. The increase in copulation rate over the course of the pre-laying period in kittiwakes (Helfenstein, Tirard, et al., 2004) and other birds (Birkhead & Moller, 1992), as well as postcopulatory sperm ejections, have been suggested to be two behavioral strategies that can prevent fertilization by aged sperm (Wagner et al., 2004; White et al., 2008). Here, we showed that these two strategies are preferentially used by more genetically similar pairs, probably as an adaptation to limit the increasing reproductive costs of old sperm with inbreeding. Similar parental modulation of the effects of inbreeding has been suggested in the Japanese quail (*Coturnix japonica*) and the burying beetle (*Nicrophorus vespilloides*), where inbreeding effects are reduced when females allocate more resources to their offspring (Ihle et al., 2017; Pilakouta, Jamieson, Moorad, & Smiseth, 2015; Pilakouta & Smiseth, 2016). This plasticity in behaviors according to genetic distance implies that individuals can assess their genetic similarity to their mate. In a large range of species, genetic similarity is assessed using odor cues (Charpentier, Crawford, Boulet, & Drea, 2010; Leclaire et al., 2017; Parrott, Ward, & Temple-Smith, 2007; Radwan et al., 2008). However, although kittiwake odors do vary with genetic relatedness (Leclaire et al., 2012), the ability of kittiwakes to use this cue has not yet been explored.

In addition to mating behaviors, other strategies based on parental phenotypic traits may modulate the reproductive consequences of the interplay between inbreeding and sperm aging. For example, parental age, i.e. the pre-meiotic senescence of the diploid organism, has been suggested to heighten inbreeding effects (Fox & Reed, 2010) and sperm susceptibility to sperm aging (Paul & Robaire, 2013; Risopatron et al., 2018; Zubkova & Robaire, 2006), and might thus modulate the costs associated with their interaction. In *D. melanogaster*, the reproductive

costs of the interaction between inbreeding and sperm aging were modulated by parental age, with young parents suffering higher costs than older ones (Tan et al., 2013). This may be explained by differential resource allocation into eggs between old and young parents (Beamonte-Barrientos, Velando, Drummond, & Torres, 2010; Bogdanova, Nager, & Monaghan, 2006; Ihle et al., 2017). If parental age modulates the reproductive costs of the interaction between inbreeding and sperm aging in kittiwakes, we would predict breeders to plastically adapt their behavior in response to both age and within-pair genetic similarity. Female kittiwakes may be more likely to use the behavioral strategies preventing fertilization by aged sperm (i.e. avoidance of precocious copulations, and sperm ejection after precocious copulations) when paired with an old, genetically similar, male.

Our results raise also questions about the role of the interaction between sperm aging and inbreeding in the strategies displayed by polyandrous species. For instance, when inbreeding interacts with sperm age to decrease fitness, we expect female cryptic preference for genetically dissimilar males to vary with sperm age. Females may not necessarily avoid inbreeding when inseminated with fresh sperm or they may bias fertilization towards the freshest sperm independently of males' genetic characteristics (C. Gasparini et al., 2018). The existence of such an interaction might partly explain why cryptic inbreeding avoidance is not ubiquitously found in nature (Mongue, Ahmed, Tsai, & de Roode, 2015) and emphasizes the importance of controlling for sperm age (or sperm quality) when testing for cryptic female sperm choice (Denk, Holzmann, Peters, Vermeirssen, & Kempenaers, 2005).

An unresolved question is which sex is responsible for the behavioral patterns observed in our study. In kittiwakes, males and females may have a common interest in avoiding fertilization by old sperm because they share the same reproductive success (Kvarnemo, 2018). However, sexual conflict over mating behavior can also arise in genetically monogamous

species because costs and benefits associated with these behaviors may differ between sexes (Helfenstein et al., 2003; White, 2008). For instance, repeated copulations increase the likelihood of sexually-transmitted pathogen transmission (Sheldon, 1993), especially in females (Van Dongen et al., 2019; White, 2008; White, Mirleau, et al., 2010). Therefore, although, in kittiwakes, both males and females have some control over copulations (White, 2008), the observed absence of seasonally early copulations in more genetically similar pairs (Figure 2A, B) is likely due to an absence of solicitations of copulation by females, who might suffer from repeated copulations more than males. Additionally, males also have some control over sperm ejection, which, despite being a female behavior, can be prevented when males remain on the female's back after insemination (Helfenstein et al., 2003).

Our results suggest that sperm aging can exacerbate the deleterious effects of inbreeding in a vertebrate and that individuals can plastically adapt their behavior in response to these selective pressures. Such best-of-a-bad job strategy might allow monogamous species to avoid fitness costs associated with delayed reproduction in a given breeding season (Cam, Monnat, & Hines, 2003). Overall, our study highlights the selective pressures sperm aging and inbreeding may exert on the evolution of reproductive behavior.

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## SUPPLEMENTARY MATERIAL

*Results – additional information*

Wearing a control ring did not prevent males from inseminating their mate. To control for any potential effects caused by the ring itself on reproductive performance, we investigated how duration of the control ring affected three proxies of fitness (i.e. hatching success and chick condition and tarsus length at hatching). Statistical analyses and models were the same than for the experimental ring (see methods in the main manuscript). Hatching success was not significantly related to any of the parameters ($n = 43$, all $p > 0.32$). Body condition at hatching was not significantly related to any of the parameters ($n = 32$, all $p > 0.26$) except egg rank ($\chi 12 = 5.96$, $p = 0.01$), A chicks being in better condition than B chicks. Finally, tarsus length at hatching was not significantly associated with any of the parameter ($n = 32$, all $p > 0.10$).

# DISCUSSION AND PERSPECTIVES

In this thesis, I found evidence that kittiwakes were able to adjust some reproductive decisions depending on the prospective genetic quality of their offspring. Specifically, these investigations first revealed positive associations between MHC-II functional diversity and fitness-related traits in female chicks, but not in male chicks. Accordingly, parents with functionally similar MHC-II, that were more likely to produce chicks with low MHC-II-diversity, overproduced sons, suggesting an MHC-dependent adaptive adjustment of sex ratio. Second, I found evidence that genome-wide genetic similarity between mates decreased egg hatchability when the fertilizing sperm was old, but not when it was young and, accordingly, genetically-similar pairs performed behaviors allowing avoidance of fertilization by old sperm. This work is a first step in the identification of the selective agents shaping fitness and reproductive decisions in kittiwakes. Experimental studies are needed to better identify these selective agents and future studies should consider other reproductive strategies.

*MHC-diversity and tick resistance*

MHC-parasite associations can be difficult to interpret in natural populations as individuals are generally sampled once. By monitoring tick infection over the course of the nestling stage, I found that one rare MHC supertype, SUP2, was associated with reduced number of ticks but not with the probability to be infected, indicating increased quantitative resistance against ticks. I also found that high functional MHC-II-diversity did not prevent tick infection nor limit the number of ticks, but that it conferred rapid clearance of tick infection in female chicks. As SUP2 was not more present in MHC-II-diverse individuals, nor conferred the same advantages than MHC-II-diversity against ticks, the association between MHC-II-diversity and tick clearance might indicate a direct effect of functional MHC-II-diversity on a single parasite species. MHC-

diversity has been shown to be advantageous against a single parasite species in the wild, possibly because it allows recognition and presentation of more antigens from this parasite (Sin et al., 2014 and references therein). However, most single infection experiments in the laboratory did not find any advantage of MHC-diversity in terms of parasite resistance (reviewed in Apanius et al., 1997). It seems more likely that functional MHC-II-diversity was advantageous against ticks in female kittiwakes because it conferred them increased resistance against multiple parasites. This would have made females in better health or physiological condition, and thus with enough resources to mount an immune response against ticks (i.e. vicious circle between bad condition and infection; Beldomenico et al., 2008). The next step would be to discriminate between these two explanations by experimentally removing all parasites except ticks from some nests. This experiment would allow to test whether high functional MHC-II-diversity increases female resistance against ticks in absence of other parasites. It would also help testing whether increased tick resistance partly explains the fitness advantages of MHC-II-diversity in female chicks, although selectively removing ticks from some nests might be a simpler way to address this question.

*MHC-fitness associations and sex*

A vast majority of MHC-fitness studies overlooked that, within a population, individuals should not benefit from the same level of MHC-diversity because they are not equally exposed to parasites or equally capable of mounting an immune response. In this thesis, I found evidence that high MHC-diversity increased survival in female chicks hatched in second, and improved growth rate and tick clearance in females, whereas I detected no evidence for a selective advantage of MHC-diversity in male chicks (Chapter 1). A recent study proposed that males should benefit from high MHC-diversity whereas females should benefit from intermediate MHC-diversity because of the effects of sex hormones on immunity (Roved et al., 2017). The

authors predicted that these differences between sexes should increase with the degree of sexual selection, i.e. with male levels of immunosuppressive hormones associated with expression of dominant behaviors (intrasexual selection) or extravagant ornaments (intersexual selection). It seems unlikely that this hormonal mechanism explains that females, but not males, benefited from high MHC-diversity in juvenile kittiwakes, suggesting that other factors can shape sex-specific effects of MHC-diversity on fitness. Although further investigations are needed, a possible explanation is that female chicks are more exposed or affected by parasite infections than male chicks because of sex-differences in physiological condition, competitiveness and behavior.

Regardless of the underlying mechanisms, these findings suggest that females are under stronger selection for increased MHC-II-diversity than males in kittiwakes. The absence of selective advantages of MHC-II-diversity in males may "drag" MHC-II-diversity to levels that are detrimental to females, suggesting a sexual conflict over MHC genes (Roved et al 2017, 2018). However, selective advantages of MHC-II-diversity should ideally be examined over an individual lifetime and future studies should investigate whether the fitness consequences of MHC-II-diversity are also sex-specific in adult kittiwakes. Roved et al. (2017) predicted no or small sex-differences in fitness consequences of MHC-diversity when sexual selection is weak. Monogamous species are often considered to exhibit low levels of sexual selection but this idea is challenged by many studies (reviewed by Kvarnemo, 2018). In the genetically monogamous black-legged kittiwake, we could expect more pronounced effects of MHC-diversity on fitness in adult males than in females because males fight fiercely for breeding sites during the early breeding season (J. C. Coulson, 1968; Wooler & Coulson, 1977), which might impair their condition and may be associated with production of high levels of immunosuppressive hormones (e.g. testosterone). In contrast, female kittiwakes may benefit from higher levels of

MHC-diversity than males because females are more susceptible to sexually transmitted bacteria (Van Dongen et al., 2019). Furthermore, both sexes display vibrant red gape, whose coloration depends on food-acquired carotenoids that are known to play a role in immunity (see Appendix). Males and females may differently allocate carotenoids to coloration and immunity, thereby leading to stronger selection on MHC genes in either sex.

*The benefits of MHC-dissimilarity between mates*

Correlational associations between fitness-related traits and genetic are a useful tool to study the fitness consequences of certain genetic characteristics in natural populations but genetic effects may sometimes be confounded with environmental effects (Kruuk & Hadfield, 2007). In Chapter 1, the positive associations between fitness-related traits and chick MHC-II-diversity in females might indicate a direct effect of MHC genes on fitness via increased parasite resistance. However, these associations may also be partly explained by the fact that high MHC-II-diverse female chicks grew in a better-quality environment than low MHC-II-diverse female chicks. Adult kittiwakes may invest more energy in reproduction when paired with an MHC-dissimilar partner by providing direct benefits (e.g. food provisioning). One way to separate the post-fertilization environmental influences from genetic effects on offspring quality is to perform a cross-fostering experiment, i.e. to transfer offspring from their natal brood to a foster brood. I conducted a cross-fostering experiment based on MHC-dissimilarity between parents during the 2017 breeding season on Middleton Island, and preliminary results suggest benefits of both parental MHC-dissimilarity and high chick MHC-diversity on chick quality (Box 2). Unfortunately, 2017 was characterized by poor breeding performances of kittiwakes on Middleton Island (mean clutch size was 0.95 ± 0.07 egg and fledging success was 23%), probably because of poor foraging conditions due to an unusual warm-water event in the northeast Pacific (Hatch S. A., personal communication). The unexpected low number of two-

eggs broods reduced the sample size, making it difficult to test for sex-specific and rank-specific effects and thus requiring a second cross-fostering experiment. Cross-fostering is certainly a useful tool to study the fitness consequences of MHC genes in natural populations but, to my knowledge, no such study has been published yet.

## Box 2 Indirect and direct benefits of mating with MHC-dissimilar mates: a cross-fostering experiment

Given that food availability is an important predictor of host condition and resistance to parasites (Lochmiller, Vestey, & Boren, 1993; Love, Salvante, Dale, & Williams, 2008; Saino, Calza, & Moller, 1997), the positive associations found between fitness-related traits and MHC-II-diversity in kittiwake daughters (Chapter 2) may partly result from increased provisioning of material benefits (e.g. food) by MHC-II-dissimilar parents. To disentangle the post-laying parental influences from chick MHC-II-diversity effects on chick viability, I performed a cross-fostering experiment according to MHC-II-similarity between parents. More specifically, two-eggs broods were cross-fostered between MHC-II-similar and MHC-II-dissimilar parents, resulting in four treatments: 1) MHC-II-dissimilar foster parents with chicks produced by MHC-II-similar parents (group DPSC); 2) MHC-II-similar foster parents with chicks produced by MHC-II-dissimilar parents (group SPDC); 3) MHC-II-similar foster parents with chicks produced by another MHC-II-similar pair (group SPSC); and 4) MHC-II-dissimilar foster parents with chicks produced by another MHC-II-dissimilar pair (group DPDC). If chick MHC-II-diversity mostly explained the selective advantages found in daughters, then we should expect SPDC and DPDC daughters to perform equally well but better than DPSC and SPSC daughters.

Unfortunately, poor breeding performances of kittiwakes in 2017 on Middleton Island resulted in a low number of two-eggs broods (n = 13 SPSC, 16 SPDC, 16 DPSC and 10 DPDC), preventing the test of sex- or rank-specific effects of chick MHC-II-diversity on fitness-related traits. Here, I present preliminary results of the effects of the experimental treatment on mortality, immune response and tick resistance in kittiwake chicks during the nestling stage regardless of their sex or hatching rank. Models included MHC-II-distance of biological parents and MHC-II-distance of fostered parents, both categorized as "similar" or "dissimilar", and their interaction. Identity of the parental combination was not included as a random effect in the models because it was redundant when included. Chick mortality (discrete variable) was neither associated with biological MHC-II-distance ($P$ = 0.16) nor with fostered MHC-II-distance ($P$ = 0.37) or their interaction ($P$ = 0.28). Nevertheless, graphically the data suggest that SPSC chicks died more than chicks in other experimental groups, suggesting additive costs of being low MHC-II-diverse and having MHC-II-similar parents (Figure 1A). A phytohemagglutinin (PHA) skin-swelling test was performed on 15-days old chicks to generates an inflammatory response. Chick response to PHA (skin swelling after 24 hours) was significantly associated with biological MHC-II-distance ($P$ = 0.05) but not with fostered MHC-II-distance ($P$ = 0.59) or their interaction ($P$ = 0.64). PHA response increased with biological MHC-II-distance (Figure 1B), suggesting that chick MHC-II-diversity better explained chick innate immune response than the parental environment. Finally, tick infection (discrete variable) was neither associated with biological MHC-II-distance ($P$ = 0.60) nor with fostered MHC-II-distance ($P$ = 0.18), but it tended to be associated with the interaction ($P$ = 0.09). There was a trend for DPSC chicks to be more likely to be infected than DPDC chicks, suggesting additive benefits of being high MHC-II-diverse and having MHC-II-dissimilar parents (Figure 1A). The low sample size prevents any firm interpretation but these results overall suggest that

both MHC-II-dissimilar parents and high MHC-II-diversity provide selective advantages to kittiwake chicks.

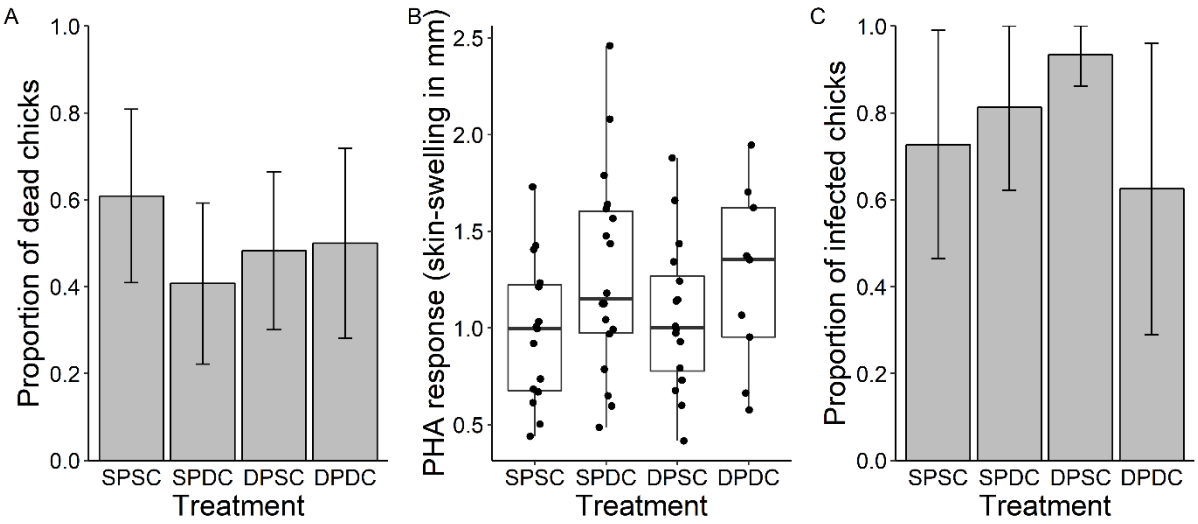



**Figure 1.** Effects of the MHC-based cross-fostering experiment on (A) chick probability to die during the nestling stage, on (B) PHA-induced immune response (skin-swelling in mm) in 15-days-old chicks and on (C) chick probability to have ticks during the nestling stage. Treatment groups are described in the main text. Error bars are based on SE.

*Assessment of MHC-similarity*

There is experimental evidence that individuals can detect MHC-similarity using odor cues in humans, rodents and lizards (Olsson et al., 2003; Radwan et al., 2008; Wedekind et al., 1995) whereas evidence in birds is currently restricted to a recent study in European storm petrels (Leclaire et al., 2017). The finding that kittiwakes produced biased sex ratio depending on MHC-II-similarity with their mate suggest that they can assess MHC-II-similarity (Chapter 2). A previous study in kittiwakes found a positive association between similarity in scent secretions chemicals and MHC-II-similarity, suggesting that kittiwakes use odors to assess MHC-II-similarity (Leclaire et al., 2014). The next step would be to test experimentally this hypothesis. I conducted such an experiment during the 2018 breeding season on Middleton Island and preliminary results suggest that adult kittiwakes can indeed identify the MHC of

other individuals via odor cues (Box 3). For now, evidence is restricted to female kittiwakes and males must be tested in the future given that sexes have been shown to express different preferences for MHC-linked odors in European storm petrels (Leclaire et al., 2017). In addition, because I tested female kittiwakes during the incubation period, I cannot exclude that results would have been different if individuals were tested during the pre-laying period, i.e. during the mate choice period. In humans, female preference for the odor of MHC-dissimilar males is reversed when females take contraceptive pills releasing pregnancy-related hormones (Wedekind et al., 1995). As important hormonal changes also occur in birds during incubation (Williams, Kitaysky, & Vezina, 2004), female kittiwakes may have reacted differently during the pre-laying period. Testing female preference for MHC-linked male odors during the mate choice period should help addressing these uncertainties. Another interesting avenue would be to test cross-fostered chicks (see Box 2) when they reach adulthood to explore whether kittiwakes recognize the MHC of other individuals via odor cues by using themselves (self-inspection) or their close kin (familial imprinting) as a referent (D. Penn & Potts, 1998).

# Box 3 Odor-based assessment of MHC-similarity

In kittiwakes, evidence for odor-based MHC recognition is restricted to a positive association between similarity in scent-gland compounds and MHC-II-similarity (Leclaire et al., 2014). Here, I conducted an experiment to test whether female kittiwakes have the ability to assess MHC-II-similarity using olfactory cues. I assessed female kittiwake response to MHC-linked odors by presenting them a piece of nest material (i.e. a sphere of approx. 2 cm diameter) mixed with a cloacal sample collected from a male. The experiment comprised four treatments in which the odor sample (nest material and cloacal sample) came either from the mate of the tested female (Mate group, n = 25) or from a male that was functionally MHC-II-similar

(Similar group, n = 29) or MHC-II-dissimilar (Dissimilar group, n = 28) to the mate of the tested female. There was also a Control group (n = 28) in which females were presented a mix of sterile saline solution and herbaceous plants that are frequently used by kittiwakes to make their nests on Middleton Island. For the three other groups, a piece of nest material was collected from the nest of the sampled male when it was incubating its eggs. At the same moment, the sampled male was caught and its cloaca was flushed with 1 mL of sterile saline solution (White, Mirleau, et al., 2010). The nest material and cloacal samples were mixed and frozen until utilization. Each female was tested with only one type of odor sample.

To assess female response to MHC-linked odors, I took advantage of a natural behavior performed by incubating kittiwakes. When an adult (male or female) is incubating, it sometimes rearranges the nest edges by moving or removing materials that stick out. The odor sample was thus placed on the edge of the nest bowl, just under the beak of the incubating female, and the female "rearranging behavior" was then recorded for 15 minutes. Females significantly used less the dissimilar odor sample to rearrange their nest compared to the control odor sample ($P = 0.03$; Figure 1). Female kittiwakes thus avoided the odor of males that were functionally MHC-II-dissimilar to their mate. There was no significant difference between other groups ($P > 0.31$).

While a preference for MHC-dissimilar individuals has been shown in several bird species (Hoover et al., 2018; Lovlie et al., 2013; Strandh et al., 2012), the phenotypic cues used by birds to assess MHC-similarity is generally not known (but see Leclaire et al., 2017 for an exception). These results suggest that kittiwakes can assess MHC-similarity via odor cues, as shown in other taxa (Bahr et al., 2012; Olsson et al., 2003; Radwan et al., 2008). Because low MHC-II-diverse daughters are less fit, kittiwakes should preferentially pair with MHC-II-dissimilar individuals. Future studies should test whether kittiwakes prefer the odor of MHC-

II-dissimilar individuals during the mate choice period (i.e. the pre-incubation period). A similar experimental method may be performed on non-incubating adults. Because nest material is impregnated with the odors from two individuals, it would be better to use a different type of sample to present odors. However, new objects like cotton swabs elicited a neophobic behavior in kittiwakes. It may thus be difficult to present single-individual odors to test MHC-preferences in kittiwakes.

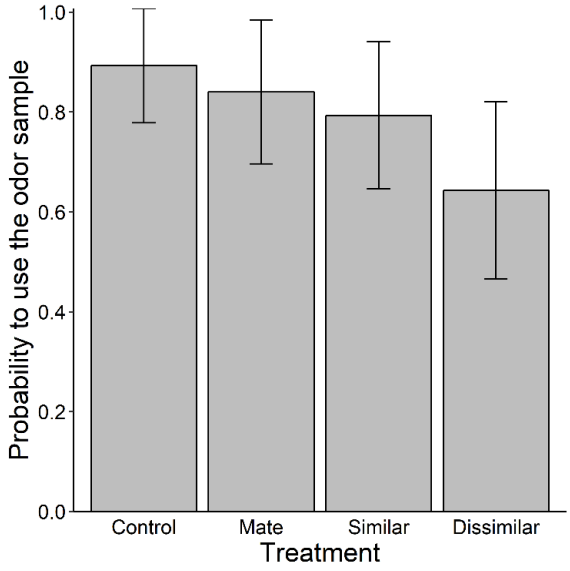



**Figure 1.** Probability that female kittiwakes used the odor sample to rearrange their nest according to the origin of the sample. The odor sample (nest material mixed with a cloacal sample) could come from the mate of the tested female (n = 25) or from a male that was either MHC-II-similar (n = 29) or MHC-II-dissimilar (n = 28) to the mate of the tested female. The control consisted in a mix of sterile saline solution and herbaceous plants (n = 28). Error bars are based on SE.

*MHC-similarity and reproductive decisions*

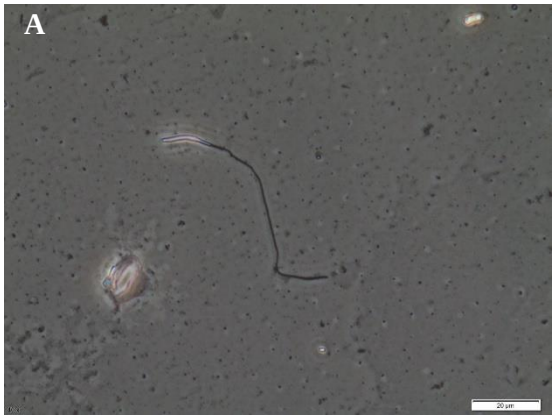
While female kittiwakes are suggested to assess their mate MHC-II-similarity to adjust sex ratio in the progeny (Chapter 2), kittiwakes might use such assessment during other reproductive decisions. A straightforward strategy to avoid fitness costs associated with production of low MHC-diverse daughters would be to preferentially pair with MHC-II-dissimilar individuals, as shown in a wide range of species (Bahr et al., 2012; Huchard et al., 2013; Santos et al., 2017), including seabirds (Hoover et al., 2018; Strandh et al., 2012). The finding that MHC-II-dissimilar kittiwake pairs overproduced sons suggests that highly MHC-II-diverse males have fitness advantages, possibly later in life during breeding (Chapter 2). Reproducing with MHC-

II-dissimilar individuals may thus improve quality of both daughters and sons in kittiwakes. MHC-dependent post-pairing, pre-fertilization reproductive strategies are much less studied and are virtually restricted to polyandrous and polygynous species, i.e. to species where males and/or females reproduce with more than one mate. In monogamous species, mates could counterbalance the detrimental effects of suboptimal pairing by improving offspring condition as it might compensate a low genetic quality (Ihle et al., 2017; Pilakouta et al., 2015). To do this, males could increase sperm quality as it is known to improve offspring viability (Immler et al., 2014). To test this hypothesis, I collected sperm samples during the 2018 pre-laying period on Middleton Island and preliminary results indicate that sperm quality increased with MHC-II-dissimilarity between mates (Box 4). Kittiwake males thus seem to invest less in sperm quality when paired with an MHC-II-similar female, possibly to lower the costs of sperm investment in a suboptimal mate.

MHC-dependent pre-fertilization reproductive strategies do not allow differential investment among offspring and may thus be suboptimal if fitness consequences of MHC-diversity are modulated by sex or hatching order. In kittiwakes, MHC-II-similar pairs could use other post-fertilization reproductive strategies than sex ratio adjustment to compensate the fitness costs of producing low MHC-II-diverse daughters, especially in second position of the laying sequence. By provisioning more resources (e.g. lipids, proteins) or specific components (e.g. antioxidants) to female embryos during egg formation, females paired with an MHC-II-similar male may improve condition of their daughters during the nestling stage, thereby increasing their capacity to fight parasites (Krist, 2011; Watson, Salmon, & Isaksson, 2018). Similar strategies may exist after hatching, with increased food provisioning of daughters by MHC-II-similar parents for instance.

# Box 4 Males paired with MHC-dissimilar females produce better-quality sperm

Males are expected to modulate their investment in ejaculate quality depending on female quality (Parker & Pizzari, 2010; Wedell, Gage, & Parker, 2002). When males mate with more than one female, we could expect them to strategically increase sperm allocation when mating with attractive females. Specifically, males have been shown to invest more in ejaculate quality when in presence of an MHC-dissimilar female in red junglefowls and horses (Burger et al., 2015; Gillingham et al., 2009; Jeannerat et al., 2018), possibly to increase fertilization success. In contrast, when males mate with only one female, they may be more likely to produce better-quality sperm when mating with a relatively low-quality female to counterbalance the detrimental effects of suboptimal pairing. Here I collected sperm samples on kittiwake males to test whether sperm quality depended on MHC-similarity between mates. I predicted that males should increase sperm quality when paired with an MHC-II-similar female to improve condition of low-MHC-II-diverse daughters as it might compensate their low genetic quality. Sperm samples were collected on 21 kittiwake males during the pre-laying period in 2018 on Middleton Island by using a simple massage technique described by Humann-Guilleminot et al. (2018). A droplet of the ejaculate was smeared with formalin on a glass slide and the first forty spermatozoa observed on the slide were categorized as normal or abnormal using a microscope (x400 magnification; Figure 1A). Spermatozoa were categorized as abnormal when deformed, fragmented or missing a piece (head, midpiece or flagellum). Contrary to prediction, the percentage of normal spermatozoa within an ejaculate significantly increased with MHC-II-distance between mates ($P < 0.001$; Figure 1B). Kittiwake males thus seem to invest less in sperm quality when paired with an MHC-II-similar female.



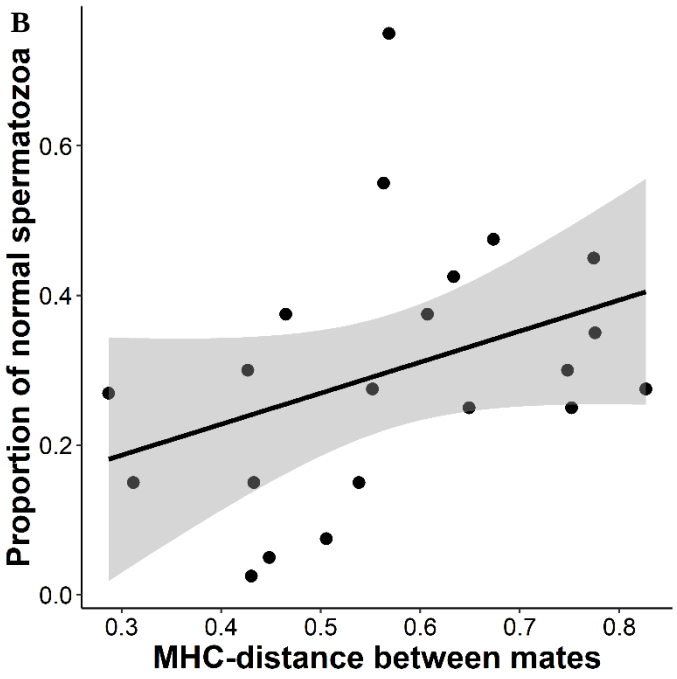




**Figure 1. A.** Picture of a normal kittiwake sperm (x400 magnification). **B.** Proportion of normal spermatozoa produced by kittiwake males according to MHC-distance with their mate. The shaded area corresponds to 95% confidence intervals.

While costs of sperm production have long been thought to be virtually inexistent, there is now compelling evidence that sperm production is time and energy consuming (Parker & Pizzari, 2010; Wedell et al., 2002). Kittiwake males may invest less in sperm quality to save energy when paired with a suboptimal female, i.e. an MHC-II-similar female. This may be advantageous in long-lived species, like kittiwakes, where animals should balance their current investment in reproduction against their investment in future reproductive events (Erikstad, Fauchald, Tveraa, & Steen, 1998). Non-exclusively, increased sperm quality may have negligible effects on offspring condition and may thus not compensate low genetic quality of offspring produced by MHC-similar parents. Male kittiwakes have a high percentage of abnormal sperm, as expected in absence of sperm competition (van der Horst & Maree, 2014), and a small number of viable spermatozoa might be enough to fertilize the female with good-quality sperm. To go further, future studies should investigate the fitness consequences of sperm quality and collect other measures of sperm quality (e.g. survival, velocity, DNA fragmentation) and also ejaculate, non-sperm, quality (e.g. seminal fluid proteins).

*Conclusion*

Overall, this thesis provides evidence that kittiwakes flexibly adapt some reproductive decisions in response to within-pair genetic similarity at key functional genes and over the whole genome. These reproductive decisions can be viewed as best-of-the-bad-job strategies allowing compensation of the detrimental consequences of suboptimal pairing (e.g. with MHC-II-similar mates). Similar strategies are likely to exist in other monogamous species, and more generally in species where individuals face constraints in choosing within a limited pool of potential mates. Importantly, future studies interested in the fitness consequences of MHC-diversity should consider any trait (e.g. sex) expected to modulate exposure or immune responses to parasites and should fully appreciate the complexity of the life history of their model species before predicting how such traits influence MHC-fitness associations. Not taking these traits into account may lead to a misunderstanding of the evolution of reproductive strategies.

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# APPENDIX

# Red coloration varies with dietary carotenoid access and nutritional condition in kittiwakes

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**ABSTRACT**

Carotenoid-based ornaments are common signaling features in animals. Although the mechanisms that link color-based signals to individual condition is key to understanding the evolution and function of these ornaments, they are most often poorly known. Several hypotheses have been posited. They include (i) the role of foraging abilities on carotenoid acquisition and thereby carotenoid-based ornaments, and (ii) the role of internal processes linked to individual quality on the allocation and conversion of carotenoids in integuments. Here we tested the influence of dietary carotenoid access vs. internal process on gape coloration in black-legged kittiwakes (*Rissa tridactyla*). This seabird displays vibrant red gape, whose coloration varies with individual quality in males and is due to the deposition of red ketocarotenoids, such as astaxanthin. We decreased hydroxy- and ketocarotenoid levels in plasma, but increased efficiency in internal processes linked to nutritional condition, by supplementing breeding males with capelin, a natural energy-rich fish prey. We found that, despite having lower carotenoid levels in plasma, supplemented birds developed redder coloration than control birds, but only in the year when dietary levels of astaxanthin in the natural diet was low. In contrast, in astaxanthin-rich years, supplemented males had less-red gape than unsupplemented birds. These results suggest that inter-individual differences in internal processes may be sufficient to maintain the honesty of gape coloration under conditions of low dietary astaxanthin levels. Nonetheless, when inter-individuals variations in dietary astaxanthin levels are elevated (such as in the crustacean-rich year), carotenoid access seems a more limiting factor to the expression of gape coloration than internal processes. Our study revealed therefore a complex mechanism of gape color production in kittiwakes, and suggests that the main factor maintaining the condition-dependency of this ornaments may vary with environmental conditions and diet composition.

*Keywords:* Ketocarotenoid, astaxanthin, zeaxanthin, bare part, gull

## INTRODUCTION

The yellow, orange or red coloration of numerous bird species results from the deposition of carotenoid pigments into integuments (McGraw, 2006). These colorations are textbook examples of sexual selection and have repeatedly been shown to depend on condition and health, and thus to play pivotal roles as honest signals of individual quality (Hill, 2006b; McGraw, 2006; Moller et al., 2000; Pérez-Rodríguez et al., 2013; Svensson and Wong, 2011). However, although special interest has been paid to elucidate the proximal sources of their variability (García-de Blas et al., 2016; Hill, 2006a; Simons et al., 2012), the mechanisms maintaining their honesty are not fully understood (Koch and Hill, 2018; Svensson and Wong, 2011; Weaver et al., 2017).

The earliest hypothesis explaining how carotenoid-based coloration relates to individual quality posits that the expression of full color is limited by dietary carotenoid access ("the foraging hypothesis"; Endler, 1980; Hill, 1992). Birds cannot synthesize carotenoids de novo, and thus must acquire carotenoids in their diet. According to this hypothesis, only individuals with good foraging ability would be able to obtain sufficient carotenoids to develop full coloration. Accordingly, in siskins (*Carduelis spinus*), blue tits (*Cyanistes caeruleus*) and brown booby (*Sula leucogaster brewsteri*), carotenoid-based coloration seems related to foraging skills (Garcia-Navas et al., 2012; Michael et al., 2018; Senar and Escobar, 2002). In addition, experimental provisioning of carotenoids consistently produces changes in coloration (Hill, 2006a; Koch et al., 2016; Simons et al., 2012), indicating that dietary carotenoid availability can limit color expression. Nonetheless, a few studies showed also that inter-individual variations in carotenoid-based coloration persist under uniform diet (Karu et al.,

2007; McGraw and Hill, 2001), suggesting that dietary factors may not be the only determinants maintaining the honesty of carotenoid-based coloration. Therefore, although the importance of dietary carotenoid access in maintaining the honesty of carotenoid-based coloration in wild birds has been debated for decades, it remains largely unresolved (Koch and Hill, 2018).

Other studies suggested that carotenoid-based colors are limited by internal processes, rather than by dietary carotenoid access (Hudon, 1994; Lozano, 1994). First, the trade-off hypothesis suggests that, because carotenoids may act as immunostimulants and antioxidants (Blount et al., 2003; Chew, 1993; Mcgraw and Ardia, 2003; Young and Lowe, 2001), only individuals with a strong immune system and effective antioxidant functions can allocate sufficient carotenoids away from these critical physiological functions to achieve full coloration (Lozano, 1994; Moller et al., 2000). However, the role of carotenoids in physiological functions is still highly contentious (Koch and Hill, 2018), and meta-analyses have revealed poor associations between carotenoid-based coloration and immune function or oxidative stress in birds (Costantini and Møller, 2008; Simons et al., 2012; Weaver et al., 2018b). Second, a more recent hypothesis suggests that the honesty of carotenoid-based coloration is maintained through its reliance on vital cellular processes (the "shared pathway hypothesis") (Hill, 2011). This hypothesis notably applies to animals that use carotenoids that they biochemically convert before deposition into integuments (Weaver et al., 2018b). For instance, most species that display carotenoid-based red coloration have to bioconvert yellow carotenoids (e.g., zeaxanthin, lutein, β-cryptoxanthin) present in the diet into red carotenoids (e.g., astaxanthin, canthaxanthin) (Hill, 1996; Weaver et al., 2018a). This metabolic conversion of carotenoids requires efficient cellular respiration (Hill, 2014; Johnson and Hill, 2013; Mundy et al., 2016), a core process with major impacts on the organism's performance (Hill, 2014; Salin et al., 2015; Salin et al., 2012). According to this hypothesis, carotenoid-based ornamentations are honest

cues of individual quality because of their fundamental dependence on the physiological state of the individual.

The debate on the proximate mechanisms maintaining the honesty of carotenoid-based signals continues, and there is still no consensus. In natural conditions, the "foraging hypothesis" is difficult to tease apart from hypotheses based on internal processes ("trade-off" and "shared pathway" hypotheses) (Linville and Breitwisch, 1997), because for most species, dietary carotenoid availability is usually confounded with food availability and thereby physiological state (Arnold et al., 2010; Ilyina et al., 2013). In this study, we therefore supplemented black-legged kittiwakes (*Rissa tridactyla*) with an energy-rich but carotenoid-poor diet to determine whether supplemented birds develop more intense coloration that unsupplemented birds. This could be consistent under either hypotheses invoking internal processes, but not under the foraging hypothesis. The black-legged kittiwake is a seabird who displays vibrant bare parts. Male gape coloration has repeatedly been shown to be positively associated with several traits related to individual condition (Blévin et al., 2014; Doutrelant et al., 2013; Leclaire et al., 2013; Leclaire et al., 2011a; Leclaire et al., 2011b), suggesting that gape coloration might be an honest signal of individual quality used in inter- or intra-sexual selection, such as mate choice, reproductive investment or competition for nesting sites. Black-legged kittiwakes feed primarily on small schooling fish (e.g., Pacific sandlance (*Ammodytes hexapterus*), capelin (*Mallotus villosus*), Pacific herring (*Clupea pallasii*)) and secondarily on ketocarotenoid-rich crustaceans such as krill and copepods (Hatch, 2013). Their integument coloration being due to red ketocarotenoids (Leclaire et al., 2015), kittiwakes seem therefore to develop redder integuments when feeding mostly on crustaceans (SL's pers. obs.). This observation suggests that, unlike most other red-colored birds that have to convert yellow

carotenoids into red carotenoids (Hill, 1996), kittiwakes can directly deposit dietary red ketocarotenoids into integuments.

To determine whether internal processes associated with nutritional condition are the main factors maintaining the honesty of gape coloration in males, we used data from an ongoing and long-term capelin-supplementation experiment. Preliminary analyses showed that, compared to a natural diet, capelin supplementation leads to lower hydroxy- and ketocarotenoid plasma levels in kittiwakes (see results). However, capelin (*Mallotus vilosus*) are lipid-rich fish prey, and their availability in the ocean is strongly correlated with kittiwake reproductive success (Hatch, 2013). Consequently, dietary supplementation with capelins have positive effects on body mass and energy expenditure rate (Jodice et al., 2002; Schultner et al., 2013; Welcker et al., 2015), and on several traits related to reproductive performance, including fledging success and chick growth (Gill and Hatch, 2002; Gill et al., 2002; Merkling et al., 2012; White et al., 2010). In addition, although the effects of capelin supplementation on adult physiological traits have not yet been determined, increased immunity and antioxidant levels are observed in supplemented chicks (Gasparini et al., 2006; Young et al., 2017). We can therefore speculate that capelin-supplemented adults are overall in better condition and less challenged by physiological trade-offs than unfed birds. In addition, capelin-supplemented birds might be able to convert carotenoids more efficiently than unfed birds, because the metabolic conversion of carotenoids requires specific enzymes and a series of oxidation steps that are likely to demand energy (Hill, 1996). Accordingly, a food-restricted diet seems to reduce the capacity of male house finches (*Carpadocus mexicanus*) to metabolically convert carotenoids (Hill, 2000). A diet rich in fish oil has also been shown to improve mitochondrial functions (Stanley et al., 2012; Yu et al., 2014), which is a key determinant in the ability to bioconvert carotenoids (Koch et al., 2017). If the condition-dependency of gape coloration in

kittiwake males is mainly due to internal processes rather than dietary carotenoid access, we thus expect capelin-supplemented males to be able to display redder gape than unsupplemented males, despite lower carotenoid levels in plasma. To test this prediction, we compared body condition, carotenoid levels and gape coloration between fed and unfed males. In addition, as a prerequisite, we ensured that, in natural condition, the honesty of gape coloration in males was maintained whatever the dietary conditions. We studied three different pre-laying seasons that differed markedly in the proportion of fish vs. crustacean in the diet, and expect, within a year, gape coloration to covary with body condition, despite being on average redder in years of high-crustacean abundance.

## MATERIALS AND METHODS

### *Study site*

The study was conducted in the 2010, 2017 and 2018 breeding seasons on a population of black-legged kittiwakes nesting on an abandoned U.S. Air Force radar tower on Middleton Island in the Gulf of Alaska (59°26'N, 146°20'W). Artificial nest sites created on the upper walls of the tower were observed from inside the building through sliding one-way windows (Gill and Hatch, 2002). This enabled us to easily capture and monitor breeders and chicks. All nest sites were checked twice daily to record events such as laying. Study birds were sexed by molecular methods (Merkling et al., 2012) or sex-specific behaviors (copulation and courtship feeding) during the pre-laying period (Jodice et al., 2000). Experiments were carried out in accordance with US laws and under permits from the US Fish and Wildlife Service and State of Alaska.

### *Food supplementation*

The amount of food available to breeders was manipulated as part of a large-scale food supplementation program in kittiwakes (Gill and Hatch, 2002). Breeding pairs were divided

into two groups: fed pairs were provided with supplemental food ad libitum whereas unfed pairs received none. The supplemental food consisted of adult Atlantic capelin (*Mallotus villosus*), an energy-rich natural prey of kittiwakes, bought frozen and thawed to ambient temperature before feeding. Supplemental feeding started on 3 May 2010, 6 May in 2017 and 6 May2018, about 4 weeks before laying (mean laying dates of A-eggs: 29 May 2010, 3 June 2017, 30 May 2018). Feeding stopped on 15 August. Feeding occurred three times daily (at 09h00, 14h00 and 17h00). During each feeding session, fish were provided singly through a plastic tube passing through the wall at each nest site (see pictures in Gill and Hatch, 2002). Feeding continued until satiation of the bird(s) present at the nest.

Unfed males (199 in total; n = 72 in 2010, 62 in 2017, 65 in 2018) and fed males (55 in total; n = 15 in 2010, 24 in 2017, 16 in 2018) were caught between 11 May and 30 May. Time between the onset of feeding and capture was mean ± se: 15 ± 1 days (range: 10-21 days) in 2010, 14 ± 1 days (range: 6-18 days) in 2017 and 9 ± 0 days (range: 7-11 days) in 2018. All males were captured prior to their mate laying the pair's first egg. At capture, birds were weighed to the nearest 5g with a Pesola® scale, tarsus length was measured to the nearest millimeter with a caliper and integument color was measured as described below. In addition, in 2010 and 2017, blood was collected from the alar vein with a 1ml syringe and a 25 gauge needle (maximum amount of blood collected: 1 ml).

*Integument color measurements*

Gape color was measured with a reflectance spectrometer (Ocean Optics USB2000), a deuterium-halogen light source (DH2000, Top Sensor System) and a 200 µm fiber optic reflectance probe held at 45° to the integument surface. Reflectance was measured using SpectraSuite software (Ocean Optics, Inc.) and in relation to dark and white (Spectralon®, Labsphere) standards. The spectrometer was re-calibrated between each bird. We measured

gape color at the intersection between the upper and lower mandibles. In 2010, color of each individual was measured once, while in 2017 and 2018, color measurements were taken three times for each individual, and the three measurements were averaged.

Reflectance spectra of the gape have peaks in both ultraviolet (UV) and visible wavelengths (Leclaire et al., 2011c). From the smoothed reflectance spectra (span=0.15), we used the R PAVO package (Maia et al., 2013) to calculate red-chroma as the relative contribution of the red spectral range to the total brightness ($R\lambda 605\text{-}\lambda 700$ / $R\lambda 300\text{-}\lambda 700$ where R indicates reflectance), and mean brightness as the mean relative reflectance between 300 and 700 nm (Montgomerie, 2006). Red-chroma and brightness were correlated (Spearman's correlation test: rho = - 0.52, $P < 0.0001$). For consistency with previous studies in kittiwakes, we also calculated carotenoid-chroma as ($R\lambda 700 - R\lambda 450$) / $R\lambda 700$ (Andersson and Prager, 2006; Butler et al., 2011; Maia et al., 2013), which is associated with fitness-related traits and circulating carotenoid levels in kittiwakes (Leclaire et al., 2015; Leclaire et al., 2011b). Gape carotenoid-chroma was positively correlated with gape red-chroma (Spearman's correlation test: rho = 0.83, $P < 0.0001$). Results using carotenoid-chroma were similar to those using red-chroma (Figs. S1, S2 and S3).

*Plasma antioxidant levels*

We analyzed carotenoid levels in the 2010 and 2017 plasma samples following the protocol described in McGraw et al. (2008). Briefly, we thawed and added 15 µl of plasma to 100 µl of ethanol in a microcentrifuge tube and vortexed for 5 s. Afterward, we added 100 µl of methyl tert-butyl ether and vortexed again for 5 s. We then centrifuged tubes for 3 min at 12,000 rpm. We transferred the supernatant to a fresh screw cap tube and evaporated to dryness with a nitrogen evaporator in a hood. Next, we resuspended the supernatant in 200 µl mobile phase, vortexed for 5 s, and injected 50 µl into a high-performance liquid chromatograph (HPLC;

Waters Alliance® Instrument, Waters Corporation, Milford, MA). We used a 5 μm Waters Carotenoid C-30 column (4.6×250 mm ID) to determine types and amounts of carotenoids present. Pigment concentrations were calculated based on external curves constructed from known amounts of purified reference carotenoids. We detected eight different carotenoids in plasma: lutein, iso-lutein, zeaxanthin, β-cryptoxanthin, astaxanthin, iso-astaxanthin, anhydrolutein, and β-carotene. Within individuals, most carotenoid levels (except β-carotene levels) were correlated to each other (Fig. S4), except in fed males in 2017, where carotenoid levels were poorly correlated to each other (Fig. S4).

*Diet analysis*

Diet samples of unfed birds were obtained in May 2010, 2017 and 2018 as regurgitations from birds captured for measurements and banding at the colony (n = 51, 67 and 40 samples respectively). Regurgitated food samples consisted of slightly-to-moderately digested masses of recently ingested prey. Samples were frozen for later identification in the laboratory. Ideally, diet composition should be expressed in terms of percent of biomass at the time of ingestion. That is not feasible for regurgitated food samples because it is difficult to separate fleshy material precisely and because of variable residence times and differential digestion in the gut (Barrett et al., 2007; Duffy and Jackson, 1986). For each prey type, we therefore used presence-absence data in each sample and expressed it as the prey type relative occurrence (as 100 x (number of samples containing prey type) / (total of prey-type identifications in all samples) (see Supplement 2 in Hatch 2013 for a discussion of this measure).

*Statistical analyses*

Fed birds were captured in a more restricted time window than unfed birds (t-tests comparing capture date of fed and unfed males in each of the three years: all $P < 0.035$). To compare fed

vs. unfed birds, we therefore excluded all unfed birds that were caught outside this restricted time window, leading to a sample size of 47 unfed males in 2010, 57 unfed males in 2017, and 14 unfed males in 2018. Effects of food-supplementation on body condition, levels of each carotenoid, and gape coloration (chroma and brightness) were assessed using LMMs (Linear Mixed Models). Food-treatment, year, date and all two-way interactions were entered as fixed effects. Bird identity was entered as a random factor. Gape chroma, gape brightness and carotenoid levels were log-transformed or boxcox-transformed to meet the normality assumption in the residuals. Body condition was estimated as the residuals of a linear regression between body mass and head-bill length performed within each sex. We used head-bill length, as is known to correlate better with mass than other structural features (Golet and Irons, 1999; Jodice et al., 2000). β-cryptoxanthin and β-carotene levels did not meet normality assumption despite transformation. We thus tested the effect of food supplementation and year on these two carotenoid levels using Wilcoxon rank-sum tests. To determine whether, in a given year, gape coloration in unfed males was a potential signal of condition and varied with carotenoid levels, we used LMMs with date, year and body condition as fixed variables and bird identity as a random effect. All statistical tests were performed with R (R Core Team, 2017). Effects were tested using maximum likelihood ratio chi-square tests, and non-significant terms were backward dropped using a stepwise elimination procedure. For all LMMs, normality and homogeneity of variance were checked visually. When heterogeneity of variance was detected, we used a specific variance structure in the model (varIdent option in the "lme" function of the "nlme" package; Zuur et al., 2009).

## RESULTS

### *Diet composition in unfed males*

In 2010, capelin and other fish species dominated the diet of unfed birds (93% of the diet as estimated by Relative occurrence, Hatch 2013), while crustaceans (amphipods) represented only 2% of the diet (Fig. 1). In contrast, in 2017, crustaceans (copepods and shrimp) contributed substantially to the diet (39%; Fig. 1), while capelin was absent and other fish prey were in low proportion (29%). In 2018, the diet of unfed birds was intermediate, and although no capelin was detected, fish represented 63% of the diet. Crustaceans represented 16% of the diet (Fig. 1).

*Effects of food-supplementation and year*

Body condition varied with the interaction between treatment and year (Fig. 2; $\chi^2 1 = 11.35$, $P = 0.0034$). In fed males, body condition did not vary with year ($\chi^2 1 = 1.38$, $P = 0.50$; Fig. 2). In unfed males, body condition was lowest in 2017 and highest in 2010 ($\chi^2 1 = 32.04$, $P < 0.0001$; Fig. 2). In 2017, fed males were in higher body condition than unfed males ($F1,76 = 30.64$, $P < 0.0001$; Fig. 2), while we detected no difference in body condition between fed and unfed males in 2010 and 2018 ($F1,61 = 2.43$, $P = 0.12$ and $F1,28= 0.53$, $P = 0.47$; Fig. 2).

Levels of astaxanthin and iso-astaxanthin (two ketocarotenoids) and levels of zeaxanthin, lutein, iso-lutein and anhydrolutein (four hydroxycarotenoids) in plasma were higher in unfed males compared to fed males (all $P < 0.001$; Figs. 3 and S5). Astaxanthin levels were higher in 2017 than 2010 ($\chi^2 1 = 9.28$, $P = 0.0023$; Fig. 3a), while zeaxanthin, lutein and anydrolutein levels in plasma were higher in 2010 than 2017 (all $P < 0.01$; Figs. 3b and S5). Iso-lutein and iso-astaxanthin levels did not vary with years ($\chi^2 1 = 0.05$, $P = 0.82$ and $\chi^2 1 = 0.52$, $P = 0.46$; Fig. S5). β-cryptoxanthin levels were higher in 2010 than 2017 (fed: $\chi^2 1 = 12.26$, $P < 0.001$; unfed: $\chi^2 1 = 26.35$, $P < 0.001$; Fig. S5) and higher in fed than unfed males in 2017 only (2017: $\chi^2 1 = 36.12$, $P = < 0.0001$; 2010: $\chi^2 1 = 0.36$, $P = 0.55$; Fig. S5). Similarly, β-carotene levels

were higher in fed males than unfed males in 2017 only (2017: $\chi^2 1 = 4.28$, P = 0.039; 2010: $\chi^2 1 = 0.26$, P = 0.61; Fig. S5).

Gape red-chroma and gape brightness varied with the interaction between treatment and year ($\chi^2 1 = 13.16$, P = 0.0014 and $\chi^2 1 = 13.62$, P = 0.0011; Fig. 4 and S6). In 2010, fed males had higher gape red-chroma and lower gape brightness than unfed males ($F_{1,59} = 6.65$, P = 0.012 and $F_{1,59} = 7.83$, P = 0.007; Fig. 4 and S6), while in 2017, they had higher brightness and lower red-chroma than unfed birds ($F_{1,79} = 9.22$, P = 0.003 and $F_{1,79} = 4.60$, P = 0.035; Fig. 4 and S6). No difference in gape red-chroma and brightness was detected between fed and unfed birds in 2018 ($F_{1,28} = 0.02$, P = 0.89 and $F_{1,28} = 0.18$, P = 0.67; Fig. 4 and S6). Gape red-chroma varied with year in both fed and unfed birds ($\chi^2 1 = 6.40$, P = 0.041 and $F_{1,115} = 49.58$, P < 0.0001), being highest in 2017 and lowest in 2010 (Fig. 4 and S6). In contrast, gape brightness varied with year in unfed males ($\chi^2 1 = 41.41$, P < 0.0001; Fig. 4 and S6) but not in fed males ($\chi^2 1 = 0.47$, P = 0.79; Fig. 4 and S6).

*Relationships between color, body condition and carotenoid levels in unfed males*

In unfed males, gape red-chroma increased with body condition in the three years studied ($\chi^2 1 = 4.13$, P = 0.042; interaction between year and body condition: $\chi^2 1 = 1.15$, P = 0.56; Fig. 5), while gape brightness did not vary with body condition ($\chi^2 1 = 2.05$, P = 0.15). In addition, gape red-chroma increased with circulating lutein levels ($\chi^2 1 = 5.37$, P = 0.021; Fig. 6a), circulating iso-lutein levels ($\chi^2 1 = 7.35$, P = 0.007) and tended to increase with zeaxanthin levels ($\chi^2 1 = 3.47$, P = 0.063). It tended also to vary with the interaction between astaxanthin levels and year ($\chi^2 1 = 3.64$, P = 0.056). Gape red-chroma increased with astaxanthin in 2010 ($F_{1,15} = 4.63$, P = 0.048), while it did not vary with astaxanthin levels in 2017 ($F_{1,31} = 0.08$, P = 0.77; Fig. 6b). Gape brightness did not vary with carotenoid levels (all P > 0.05).

## DISCUSSION

To shed some light on the main proximate mechanisms maintaining the honesty of this color signal, we investigated the factors that shape gape coloration in black-legged kittiwake males. First, in line with previous studies in this species (Blévin et al., 2014; Doutrelant et al., 2013; Leclaire et al., 2013; Leclaire et al., 2011a), we found that within a year, unsupplemented males in better condition had redder gape than unsupplemented males in poorer condition, thereby confirming the honesty of this color signal in male breeders (figure 6). Then, using both correlational and experimental observations, we detected a complex mechanism shaping gape coloration.

*Are dietary ketocarotenoids drivers of gape coloration?*

The three study years varied in environmental food composition. In May 2010, the diet of black-legged kittiwakes was mainly composed of lipid-rich capelin, while in May 2017 there was no capelin in the diet, and crustaceans such as shrimps and copepods represented a high proportion. Crustacean preys of seabirds contain high levels of astaxanthin (a ketocarotenoid), while different fish preys contain low or very low astaxanthin levels (Hipfner et al., 2010). Consequently, unsupplemented males had higher astaxanthin levels in blood in the 2017 crustacean-rich year than in the 2010 fish-rich year. This result adds evidence to the suggestion that, in contrast to terrestrial granivorous birds (García-de Blas et al., 2016), aquatic birds feeding on fish and crustaceans have the capacity to assimilate astaxanthin directly from the diet (Juola et al., 2008; McGraw and Hardy, 2006; Negro and Garrido-Fernandez, 2000).

The red-orange coloration of kittiwake integuments being mainly due to red ketocarotenoids (Leclaire et al., 2015), kittiwake gape were redder in the crustacean-rich year than in the fish-rich year (i.e., higher carotenoid-chroma and lower brightness in gape in 2017

than in 2010). Consistently, in 2018, when the kittiwake diet contained an intermediate percentage of fish and crustaceans compared to 2010 and 2017, birds had intermediate gape coloration. These results suggest that dietary ketocarotenoids, that are assimilated and present in plasma, can be deposited to the integument. They further suggest that large inter-annual variations in dietary availability of ketocarotenoids contained in crustaceans can drive inter-annual variations in gape coloration in this population of kittiwakes. Similar inter-annual relationship between dietary carotenoids and coloration in wild species have been shown, for instance, in American redstarts (*Setophaga ruticilla*), who develop redder feathers during rainy molting periods, when insect abundance and dietary carotenoid availability are higher (Reudink et al., 2015), and in Montagu's harrier (*Circus pygargus*) nestlings, who develop duller bare parts in years when voles, a carotenoid-poor prey, are abundant (Sternalski et al., 2010).

Food-supplemented males had lower ketocarotenoid levels in plasma than unsupplemented males both in 2010 and 2017. However, they developed redder gape than unsupplemented males in 2010. Their redder gape - despite lower ketocarotenoid levels in plasma - in 2010, and the lack of correlation between plasma astaxanthin levels and gape coloration within individuals observed in 2017 support the suggestion that dietary astaxanthin levels is not the main factor limiting the expression of gape coloration.

*Dietary hydroxycarotenoids as drivers of gape coloration?*

Within a year, males with higher plasma levels of zeaxanthin, lutein and iso-lutein (three hydroxycarotenoids) displayed a redder gape. In many taxa including birds, zeaxanthin and lutein are precursors of red ketocarotenoids deposited in bare parts or plumage (García-de Blas et al., 2016; LaFountain et al., 2015; McGraw, 2006; McGraw et al., 2001). These three hydroxycarotenoids were in lower levels in plasma in 2017 than in 2010, suggesting that they

are more abundant in fish than in crustaceans. Accordingly, lutein and zeaxanthin are detected in fish prey such as sand lance, capelin and herring, while they are not detected in crustaceans, like krill and copepods (Hipfner et al., 2010; Slifka et al., 2013). It could be hypothesized that males who develop redder coloration might be those with better foraging abilities and thus able to acquire higher quantities of hydroxycarotenoid-rich fish. However, a previous study in kittiwakes shows that an experimental supplementation with lutein+zeaxanthin, causing a decrease in blood astaxanthin levels, does not increase gape coloration in males (Leclaire et al., 2015). Thus, variation in dietary levels of lutein+zeaxanthin alone does not necessarily explain variation in gape coloration.

Although in natural conditions, a fish-based diet seems richer in hydroxycarotenoids than a crustacean-based diet, capelin-supplemented males had lower hydroxycarotenoid plasma levels than unsupplemented males. This difference between natural and experimental conditions might stem from supplemental capelins being less rich in carotenoids than other natural fish prey. In addition, in our study, supplemental capelins came from Iceland and Newfoundland, and were thus likely to have been caught in winter (International Council for the Exploration of the Sea, 2017), when capelin carotenoid contents is low compared to the bird breeding period (Bragadóttir et al., 2002). Freezing might also have decreased carotenoid quantity in supplemental capelins. Despite lower hydroxycarotenoid levels in plasma, capelin-supplemented males developed redder gape than unsupplemented males in 2010. This result further suggests that dietary acquisition of hydroxycarotenoids is not the main limiting factor for gape coloration.

*Internal processes as drivers of gape coloration?*

The redder gape, despite lower carotenoid levels in plasma, of food-supplemented males compared to unsupplemented males in 2010 suggests that internal factors acting somewhere between the blood and integuments may also influence gape coloration in black-legged kittiwakes. Capelin-supplemented birds, being probably in better physiological condition (albeit not in higher body condition, which might be explained by the "lean-and-fit" hypothesis (Schultner et al., 2013)), may have lower demands for immunity than unfed males, as shown in fed kittiwake chicks (Gasparini et al., 2006), and thus they may be able to allocate higher levels of the immuno-stimulating hydroxycarotenoids (Leclaire et al., 2015) into ketocarotenoid formation rather than into immunity ("trade-off hypothesis"; Blount et al., 2003; Lozano, 1994; Moller et al., 2000). Non-exclusively, fed males, being in better condition overall than unfed males, may have higher mitochondrial performance, which seems to be required for efficient carotenoid metabolic conversion ("shared pathway" hypothesis) (Hill, 2011; Weaver et al., 2018b). Disentangling these two hypotheses is challenging but some avenues have been suggested, including experimental manipulations of mitochondrial functions and the use of radiolabelled carotenoids to track carotenoid movement through the body (Bhosale et al., 2007; Koch and Hill, 2018; McGraw, 2009; Weaver et al., 2017)

*A complex interaction between internal processes and dietary carotenoid access*

In the 2017 crustacean-rich year, supplemented males were not able to develop redder gape than unsupplemented males, despite being in much better condition. When ketocarotenoids are abundant in the environment (such as in crustacean-rich years), higher efficiency in internal processes by fed birds might thus not compensate for the direct deposition of unmodified dietary ketocarotenoids into integuments by unfed males. Therefore, although differences in internal processes seem sufficient to maintain the honesty of gape coloration when the diet is poor in astaxanthin, they are not when large inter-individual variations in dietary astaxanthin levels

occur. Yet, in the 2017 crustacean-rich year, gape coloration was related to individual condition in unsupplemented males. Further studies investigating for instance how gape coloration depends on foraging abilities and diet composition are needed to evaluate whether, under a crustacean-rich diet, the honesty of gape coloration may be mainly maintained by dietary carotenoid access. Because crustaceans are ketocarotenoid-richer but energy-poorer than fish, this mechanism of honesty might have evolved only if the quantity of food acquired varies among individuals whilst the diet composition is relatively stable. However, during the pre-laying period, diet composition of kittiwakes can change drastically within a few days (Hatch, 2013). For instance, in May 2018, the diet went from being mainly based on crustaceans to being mainly based on fish, and we observed males regurgitating fish, and others regurgitating crustaceans on the same days (SL's pers obs). If good-quality individuals were the first to forage on fish, they may have acquired less dietary astaxanthin, and thereby developed less-red gape. Thus the dynamics of red-coloration needs to be studied to determine whether, during this short period of time, when the type of carotenoids ingested varies greatly among individuals, gape coloration honestly reveals the condition of the bearer.

*Concluding remarks*

We found that food supplementation, despite leading to elevated reproductive success (Gill and Hatch, 2002; Gill et al., 2002), might disrupt the honesty of integument coloration in kittiwakes. At our study site, fed birds are surrounded by unfed neighbors, and during crustacean-rich year, they may display duller gape than their neighbors despite being in better condition, and thus potentially good-quality partners. In such circumstances, females might breed with a suboptimal partner if they use gape coloration to choose their mate. More generally, while wildlife feeding is a common activity that provides an energy source to animals, it can cause

deficiency in essential nutrients like carotenoids (Tauler-Ametllller et al., 2019), and potentially change selective pressures on phenotypic traits.

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## COMPETING INTERESTS

The authors declare no competing or financial interests.

## AUTHOR CONTRIBUTIONS

Conceptualization: S.L.; Methodology: S.L., S.A.H.; Investigation: S.L., M.P.; Formal analysis: S.L.; Writing - original draft: S.L.; Writing - review & editing: V.B., M.P., P.B., E.D., S.A.H; Funding acquisition: S.L., V.B., E.D., S.A.H.

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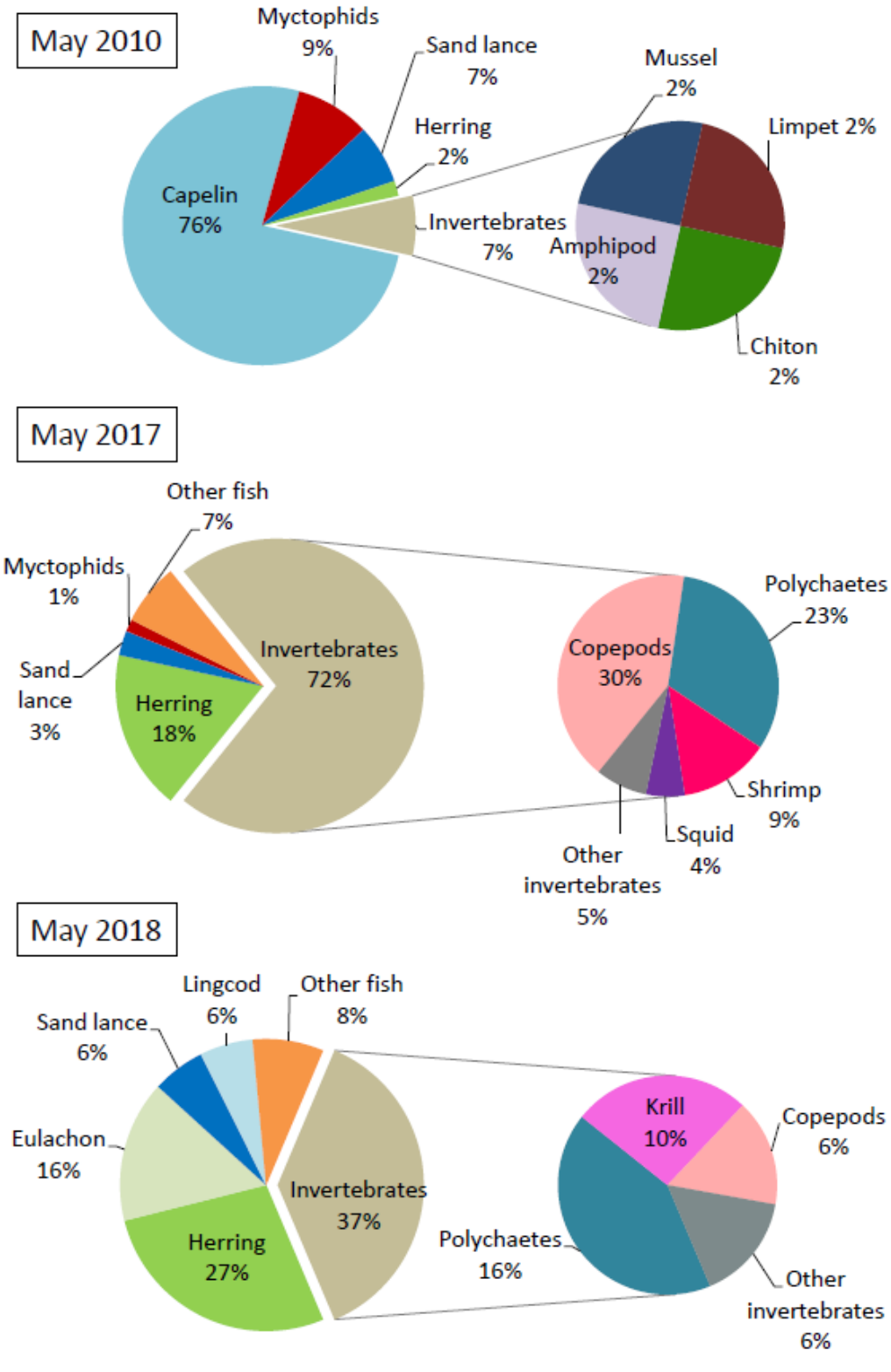


**Figure 1:** Composition of the diet of unfed kittiwakes in May 2010, 2017 and 2018. Sample sizes are 51, 67 and 40 food samples respectively.

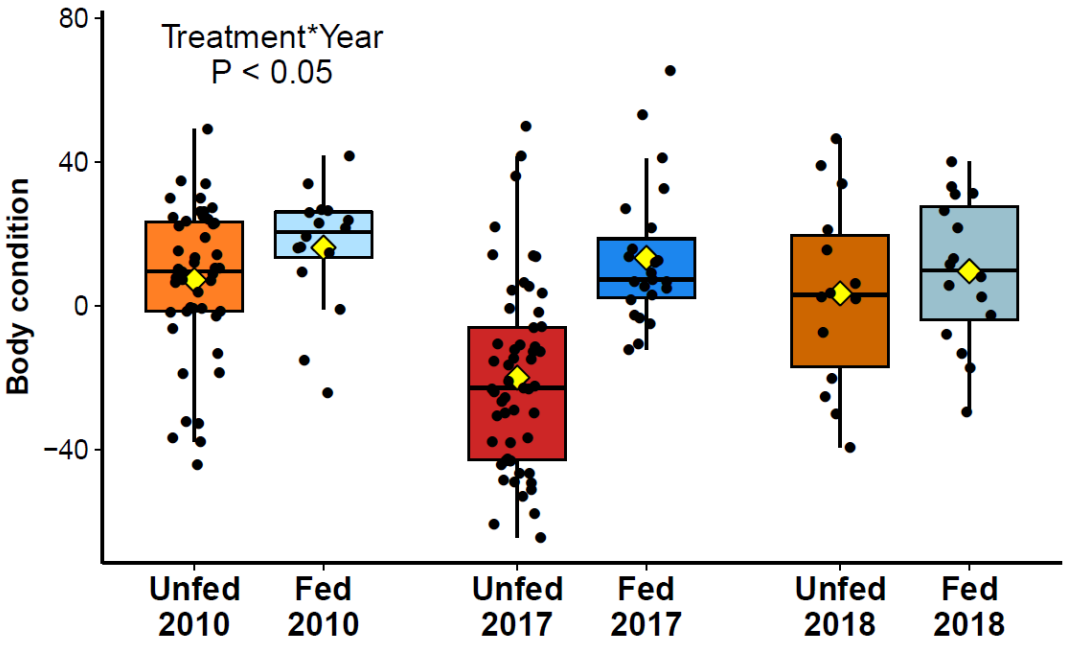


**Figure 2:** Boxplots showing body condition in unfed and fed males in 2010, 2017 and 2018. For each group, points are slightly dispersed on the x-axis to avoid overlapping. The yellow diamonds correspond to the mean.

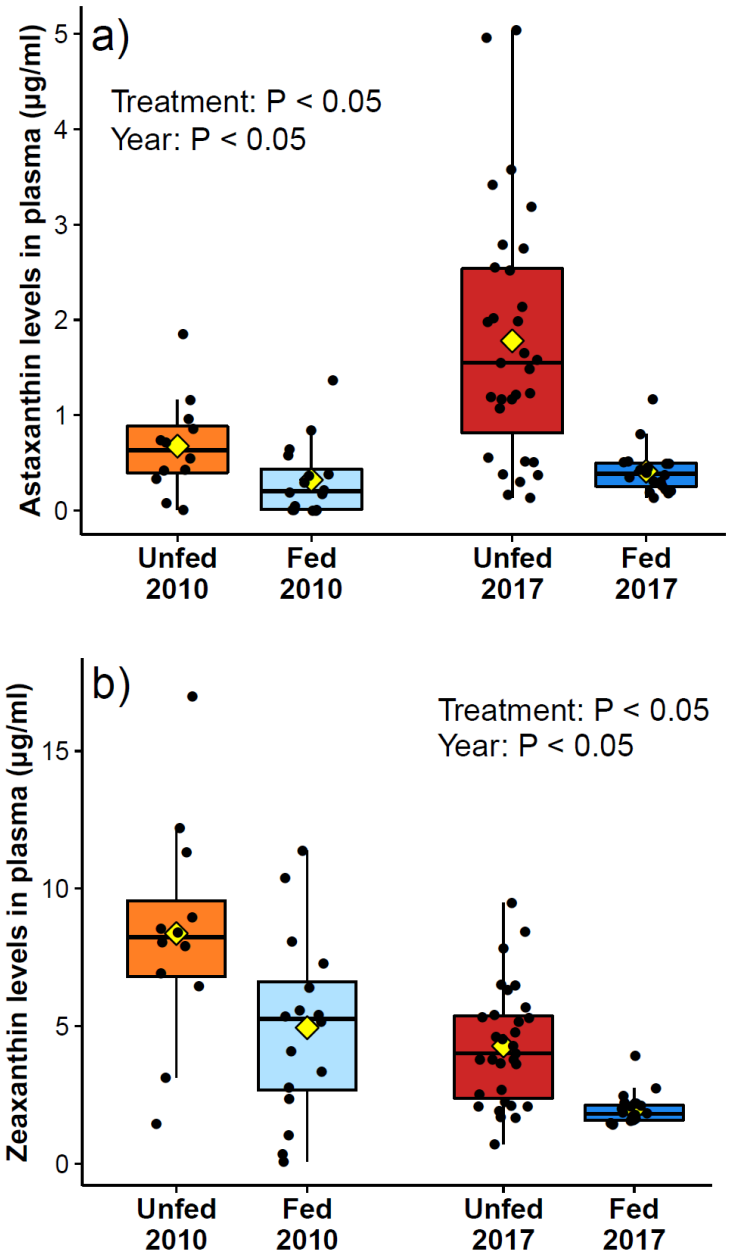


**Figure 3:** Boxplots showing plasma levels of (a) zeaxanthin and (b) astaxanthin in unfed and fed males in May 2010 and May 2017. For each group, points are slightly dispersed on the x-axis to avoid overlapping. The yellow diamonds correspond to the mean.

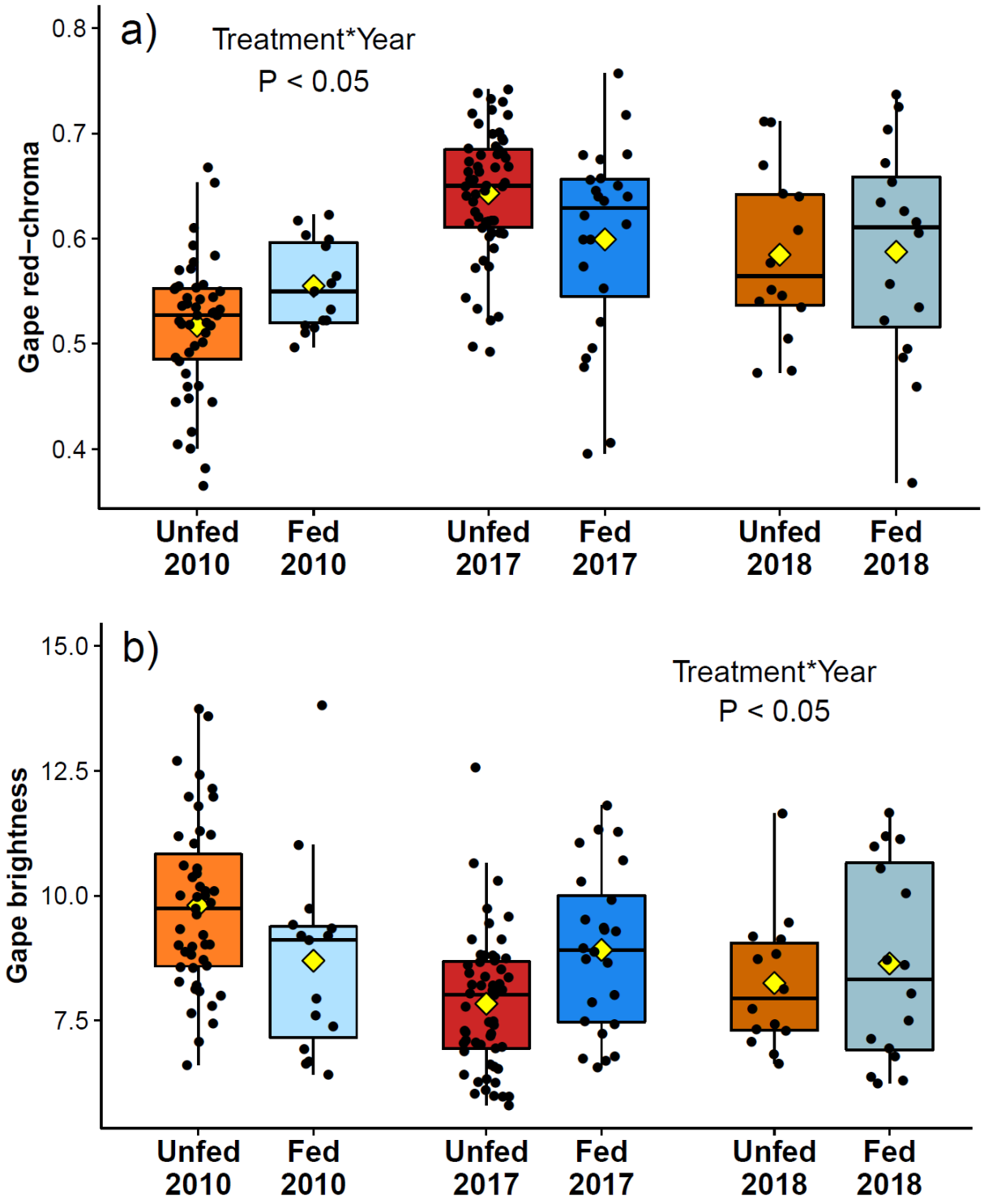


**Figure 4:** Boxplots showing (a) gape red-chroma and (b) gape brightness in unfed and fed males in May 2010, 2017 and 2018. For each group, points are slightly dispersed on the x-axis to avoid overlapping. The yellow diamonds correspond to the mean.

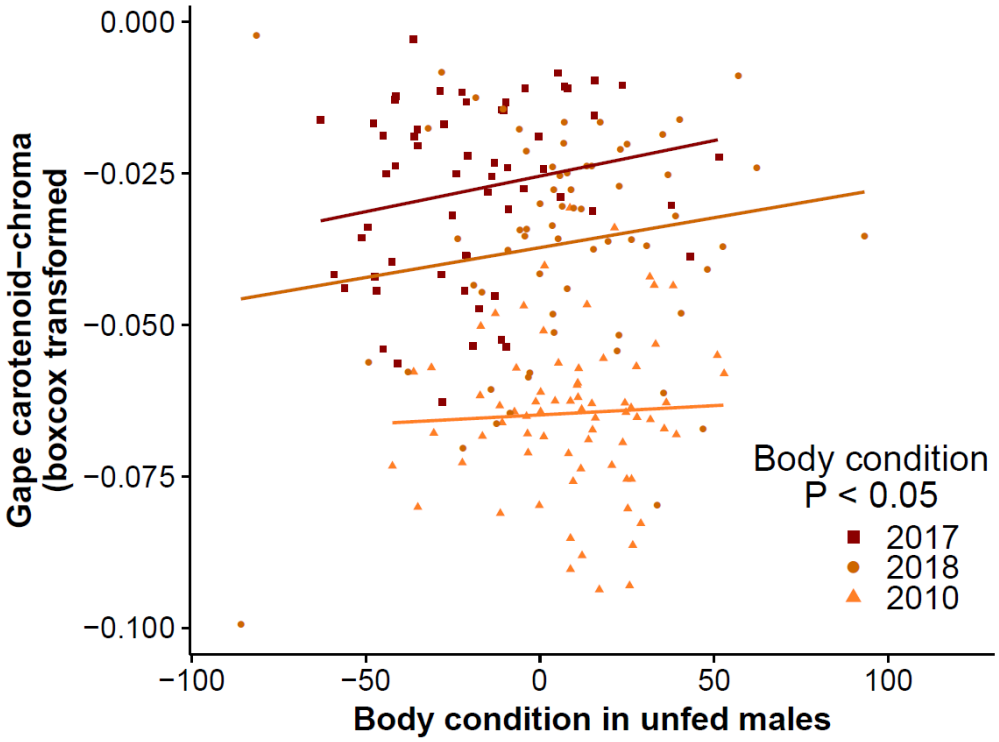


**Figure 5:** Gape red-chroma in relation to body condition in unfed males in May 2010, 2017 and 2018.

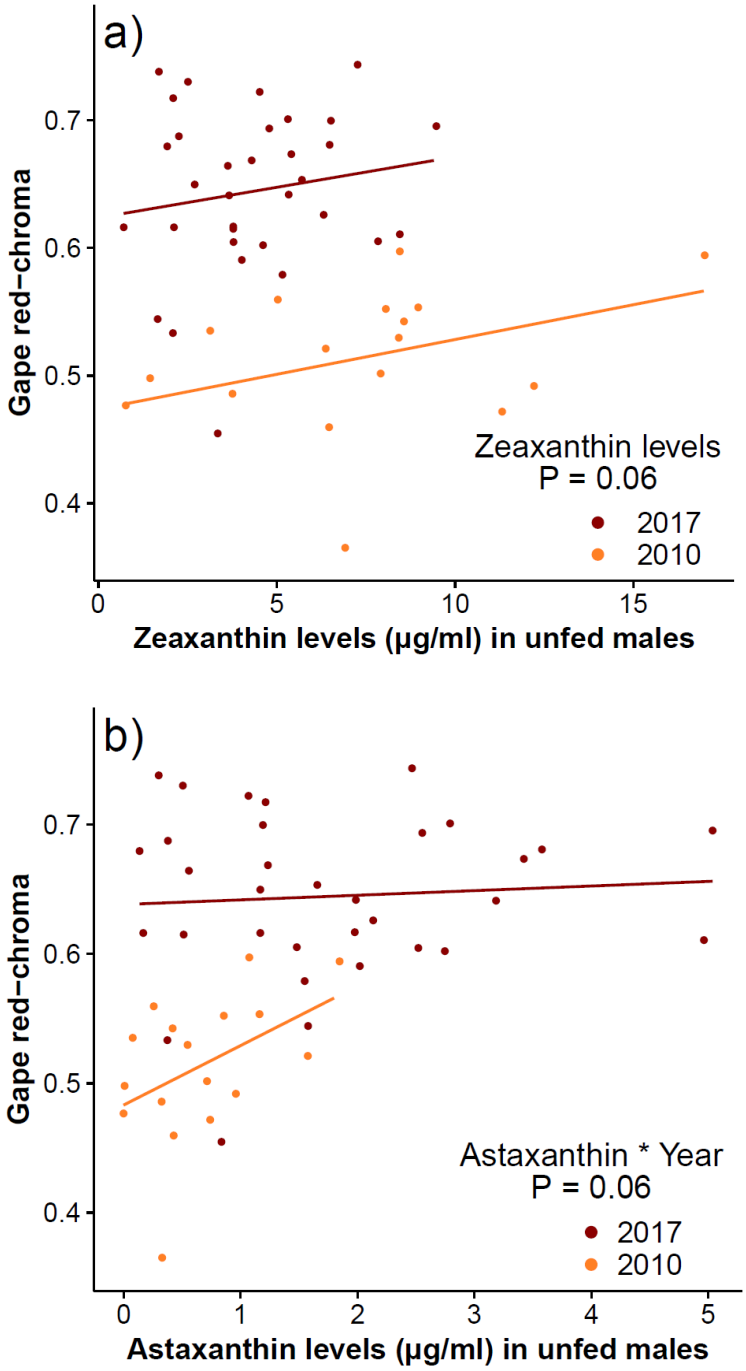


**Figure 6:** Gape red-chroma in relation to (a) zeaxantin and (b) astaxanthin plasma levels in unfed males in May 2010 and May 2017.

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**TITLE:** MHC immune genes in a monogamous seabird: fitness and reproductive strategies

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**ABSTRACT:** Parents are expected to adjust their reproductive decisions depending on the future advantages they will gain. These advantages include increased offspring fitness through acquisition of genetic benefits from mates. However, constraints may force individuals to mate with suboptimal partners. The costs of suboptimal pairing should have created selective pressures inducing the evolution of counter strategies. In this thesis, I investigated whether individuals adjust some reproductive post-pairing decisions depending on the prospective genetic characteristics of their offspring, along with the fitness consequences of these genetic characteristics, using a monogamous seabird species, the black-legged kittiwake (*Rissa tridactyla*). First, I found that chick functional diversity at major histocompatibility complex class II (MHC-II) genes, which play a pivotal role in vertebrate immunity, was positively associated with fitness-related traits in females, but not in males. Accordingly, parents with functionally similar MHC-II, that were more likely to produce chicks with low MHC-II-diversity, overproduced sons, in line with sex allocation theory expectations. Second, I report experimental evidence that genome-wide genetic similarity between mates decreased egg hatchability when the fertilizing sperm was old. In line with our expectations, genetically-similar pairs performed behaviors allowing avoidance of fertilization by old sperm. Overall, this thesis provides evidence that parents flexibly adapt some reproductive decisions in response to within-pair genetic similarity at key functional genes and over the whole genome, thereby partly compensating the detrimental consequences of suboptimal pairing.

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**KEYWORDS**: parasites; immunity; major histocompatibility complex; reproductive behaviors; senescence; odors

**DISCIPLINE**: Ecology, biodiversity and evolution

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**RÉSUMÉ :** Pendant la reproduction, les parents devraient ajuster leurs décisions en fonction des bénéfices qu'ils en tireront. La production de descendants de bonne qualité peut notamment être assurée par le choix d'un partenaire de bonne qualité génétique. Cependant, des contraintes peuvent limiter ce choix et entraîner un appariement sous-optimal, dont les coûts devraient avoir favorisé l'évolution de stratégies compensatoires après l'appariement. Dans cette thèse, je me suis intéressé aux gènes immunitaires du complexe majeur d'histocompatibilité de classe II (CMH-II) et à la consanguinité chez un oiseau marin monogame, la mouette tridactyle (*Rissa tridactyla*). Tout d'abord, la diversité au niveau du CMH-II des poussins était associée positivement à leur aptitude, mais cela n'était vrai que pour les femelles. En accord avec ces résultats, les parents avec un CMH-II fonctionnellement similaire, qui produisent des poussins peu divers au niveau du CMH-II, surproduisaient des fils, conformément aux attendus de la théorie de l'allocation au sexe. Concernant la consanguinité, elle réduisait la probabilité d'éclosion des œufs lorsque le sperme fécondant était âgé. Comme attendu, les couples consanguins exprimaient des comportements sexuels leur permettant d'éviter la fécondation par du sperme âgé. Dans l'ensemble, cette thèse montre que les parents peuvent ajuster certaines de leurs décisions reproductives en fonction de leur similarité génétique au niveau de gènes fonctionnellement importants et sur l'ensemble du génome, leur permettant ainsi de compenser en partie les coûts d'un appariement sous-optimal.

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**MOTS-CLÉS** : parasites ; immunité ; complexe majeur d'histocompatibilité ; comportements reproducteurs ; sénescence ; odeurs

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